The ‘Azirine/Oxazolone Method’ on Solid Phase

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I would like to express my sincere gratitude to

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continued and generous support.

to Eliane

Preface

This Ph.D. thesis is based on the results published or being published in international scientific journals. It is presented in four chapters corresponding to the papers in as much an unchanged form of the respective manuscripts as possible. Therefore, compounds and references are numbered independently in each chapter. An overview of the entire work is given in the following summary.

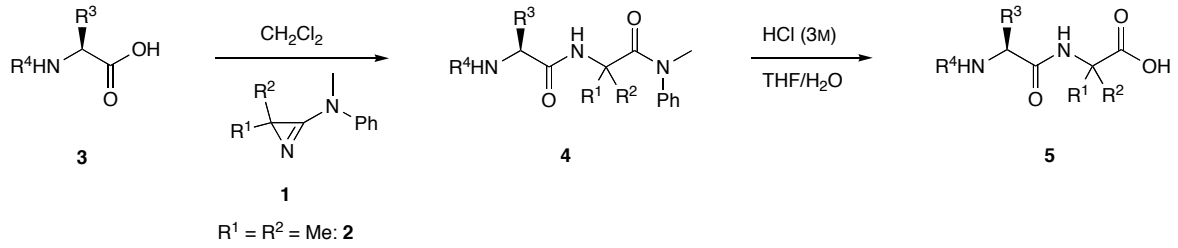
CONTENTS

1. Summary

1.1. Introduction

Peptides that contain α,α-disubstituted α-amino acids are restricted in their conformational freedom. As a consequence of the rigidity of the peptide backbone, secondary structures such as β-turns and helices are stabilized or even favored. The synthesis of such conformationally restricted peptides is a basic approach when searching for the biologically active conformation. Furthermore, these highly organized structures are responsible for the interesting biological activities of the peptaibols, a class of linear antimicrobial peptides, which contain a high number of α,α-disubstituted α-amino acids, above all, α-aminoisobutyric acid (Aib).

A useful protocol for the introduction of α,α-disubstituted α-amino acids into peptides is the 'azirine/oxazolone method', in which 2*H*-azirin-3-amines **1** are used as amino acid synthons *(Scheme 1)*. Thus, the reaction of 2*H*-azirin-3-amines, *e.g.*, the Aib synthon **2**, with an amino or peptide acid **3** proceeds smoothly and in high yield. The terminal amide bond of the resulting peptide amide **4** can be hydrolyzed selectively to give the extended peptide acid **5**. In solution-phase chemistry, the 'azirine/oxazolone method' has proven to be successful for the introduction of a variety of sterically demanding α,α-disubstituted α-amino acids into peptides, and it has found application in the synthesis of some antibiotic peptaibols or segments thereof.

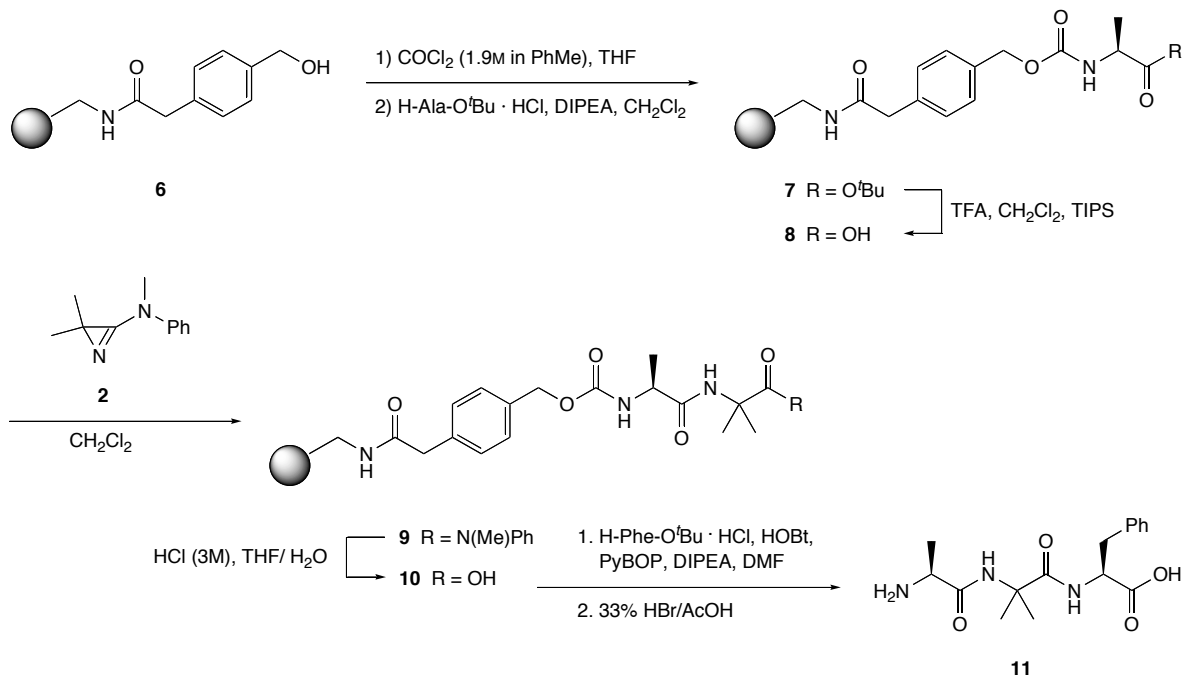


Scheme 1

Solid-phase synthesis offers rapid access to peptides without the need for the isolation of the sometimes cumbersome peptide acid intermediates. We were interested if it might be possible to combine these two features, the solid-phase synthesis and the 'azirine/oxazolone method', and apply the 'azirine/oxazolone method' to solid-phase conditions.

1.2. The 'Azirine/Oxazolone Method' on Solid Phase (CHAPTER 4)

The first questions concerned chemical reactivity: do 2*H*-azirin-3-amines react in a similar way on a solid support as in solution? For that purpose, we added 2*H*-azirin-3-amine **2** to a carboxy-polystyrene resin, which, after washing, was treated with 3M HCl. This two steps were repeated. Attenuated total reflectance (ATR) FT-IR measurements showed an alternating disappearance and appearance of the carboxy absorption band, confirming our assumptions.
In all variations of solid-phase synthesis, the linker is of crucial importance. For our purpose, the linker has to be stable during hydrolysis of the terminal amide with 3M HCl and deprotection of the ^{t}Bu ester with trifluoroacetic acid (TFA), but finally should be cleavable under conditions, not affecting the peptide. Finally, it was shown that the carbamate linker formed from PAM-resin fulfills these requirements best (*Scheme 2*).



Scheme 2

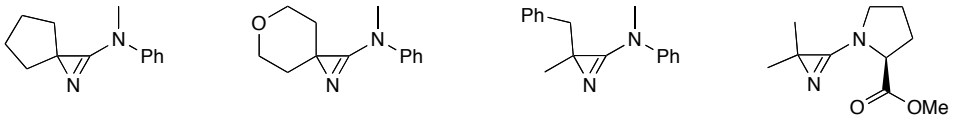
A solution of phosgene in toluene was added to PAM-resin **6** to generate a chloroformate intermediate, which was reacted with H-Ala-O^{t}Bu to afford resin **7**. Deprotection of the ^{t}Bu ester **7** with TFA led to resin **8**, which was treated with a solution of *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**2**). Unconsumed **2** could easily be recovered and re-used. Selective hydrolysis of the terminal amide with 3M HCl in THF/H_2O afforded peptide acid resin **10**. Further extension of the peptide chain could be achieved either with a ^{t}Bu protected

amino acid and a coupling reagent, *e.g.*, PyBOP, or with **2**. Cleavage from the resin was achieved with HBr (33%) in acetic acid to give the tripeptide **11**. By this method, several model peptides and short segments of peptaibols were synthesized.

1.3. Introduction of Various α,α-Disubstituted α-Amino Acids (CHAPTER 5)

It was of interest to ascertain if this method is restricted to the α-aminoisobutyric acid (Aib) synthon **2**, or if it can be extended to other 2*H*-azirin-3-amines. It was shown that the method is not limited to the Aib synthon **2**, and it was extended successfully to the 1-aminocyclopentane-1-carboxylic acid synthon **12**, the 4-aminotetrahydro-2*H*-pyran-4-carboxylic acid synthon **13** and the α-methylphenylalanine synthon **14** (*Figure 1*). Peptides with up to seven residues, of which three are α,α-disubstituted α-amino acids, have been prepared.

In contrast, the synthesis of peptides containing the poly-Aib motif was not successful, most probably due to aggregation.



12 **13** **14** **15**

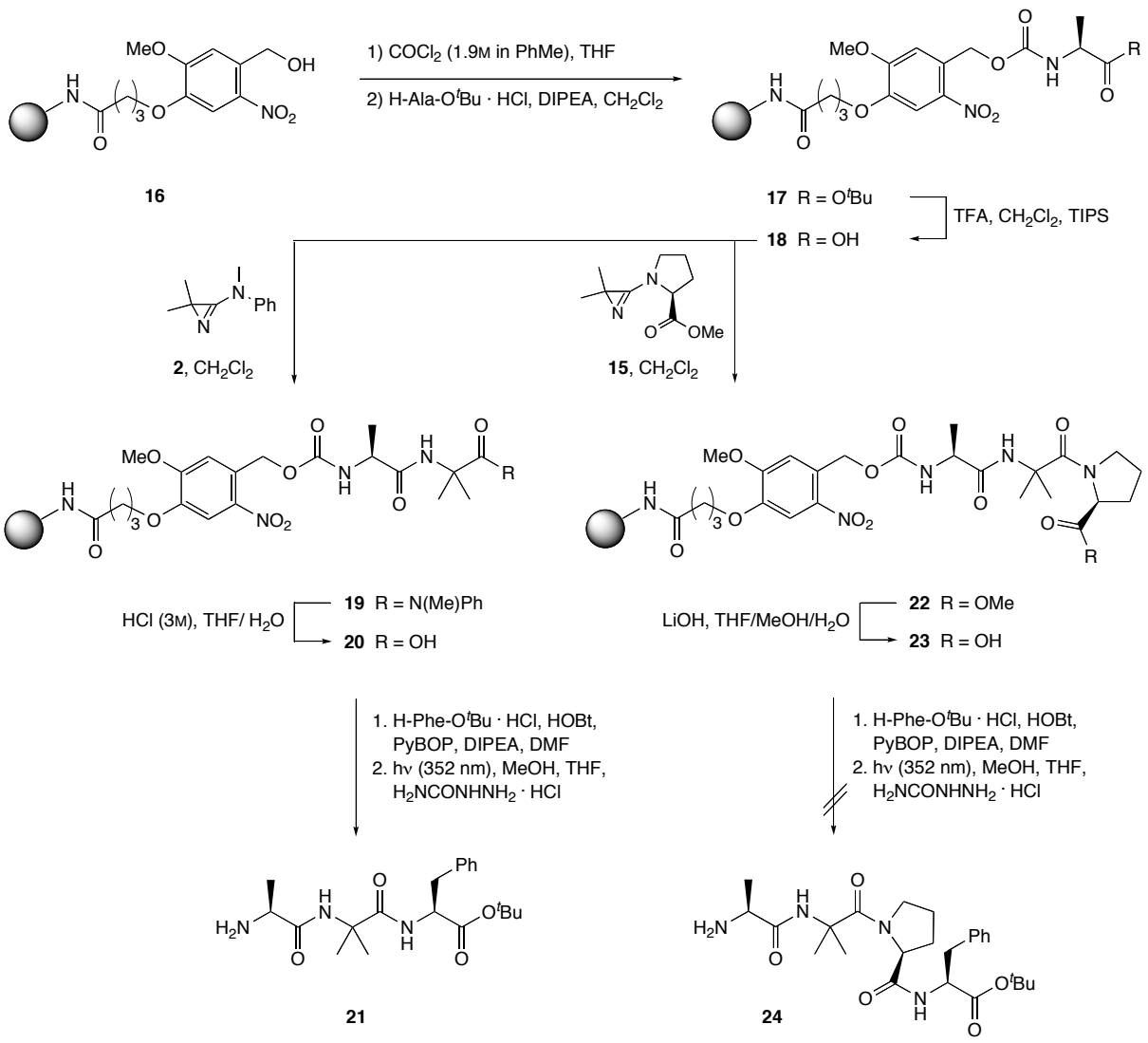
Figure 1

1.4. Introduction of the Aib-Pro Unit (CHAPTERS 6 AND 7)

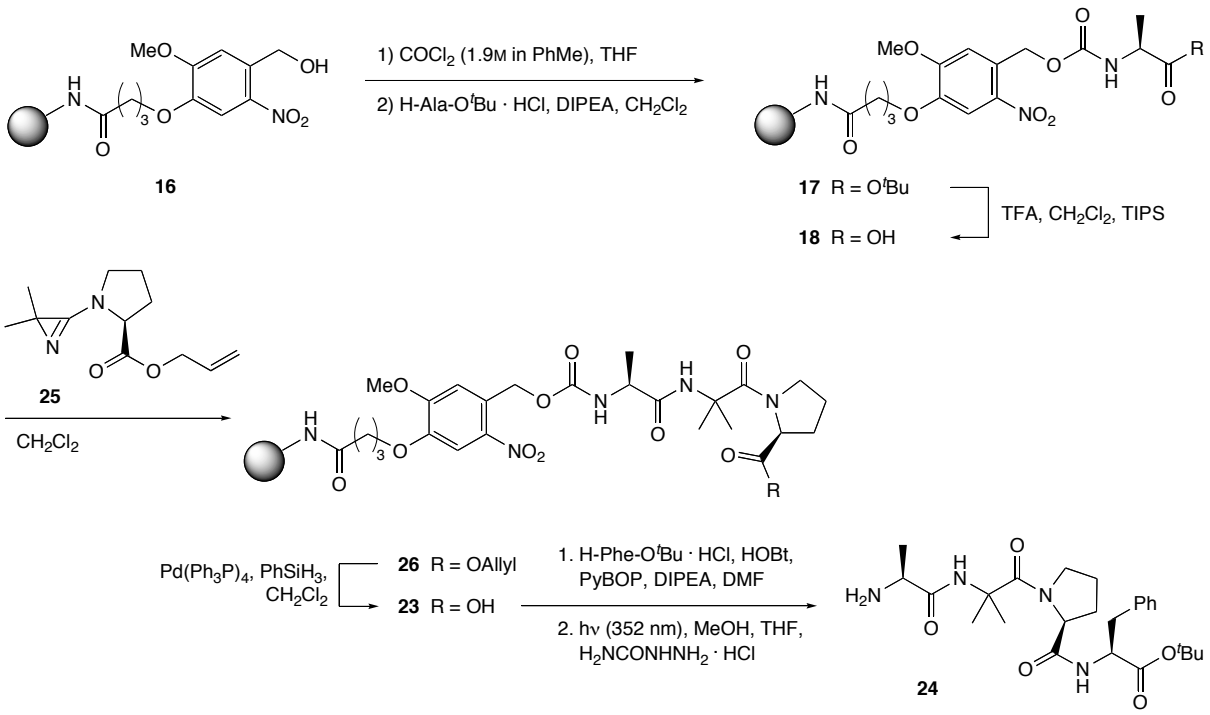
Since many peptaibols contain the Aib-Pro motif, it would be of great interest to introduce this unit by a dipeptide synthon. In solution-phase synthesis, this was indeed possible by using the dipeptide synthon **15** (*Figure 1*). However, when using **15** on solid phase, the experiments failed. The reason for this failure is the acid sensitivity of the Aib-Pro amide bond to strong acids such as HBr, we used to cleave the peptide from the resin.

While a Pd^0-labile allyl linker led not to the desired results, the use of the photolabile nitroveratryl linker **16** allowed the successful preparation of the model peptide

H-Ala-Aib-Phe-O^{t}Bu (**21**) (*Scheme 3*). But when the dipeptide synthon **15** was used to incorporate the Aib-Pro unit, the experiment failed again (*Scheme 3*). During saponification of resin **22**, we observed a considerable darkening of the resin. Hence, quenching prevented photocleavage of the peptide from the resin. This issue was addressed by the synthesis of new 2*H*-azirin-3-amines, which contain easily removable carboxy-protecting groups. Finally, we were able to prepare the phenacyl ester and the allyl ester (**25**) protected dipeptide synthons. The latter one was successfully introduced into H-Ala-Aib-Pro-Phe-O^{t}Bu (**24**) using the previously described photolinker **16** and a Pd^0-promoted procedure for the deprotection of allyl ester **26** (*Scheme 4*).



Scheme 3



Scheme 4

1.5. Applications (CHAPTER 7)

To evaluate the use of the 'azirine/oxazolone method' on solid phase, we attempted to synthesize some Aib-Pro containing peptaibol segments. H-Val-Aib-Gly-Aib-Aib-Pro-Leu-O^{t}Bu (**27**; A8 – A14 of *Trichovirin Ia*), H-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-O^{t}Bu (**28**; A1 – A6 of *Trichovirin I 1B*) and H-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu (**29**; A7 – A14 of *Trichovirin I 1B*) were prepared by using the photolinker **16**, the Aib synthon **2** and the Aib-Pro synthon **25**.

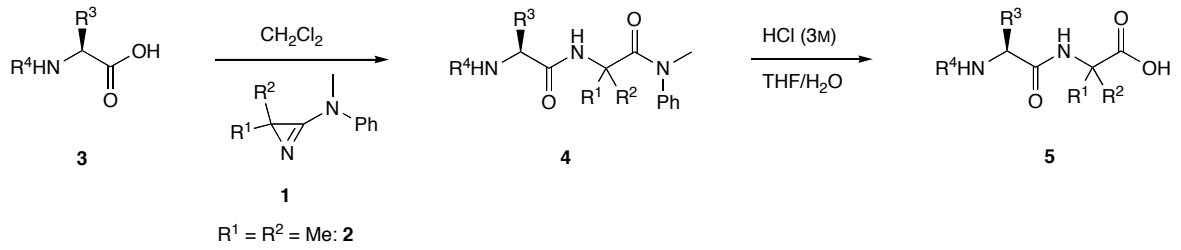
In contrast to the 'PAM/HBr-strategy' (*Scheme 2*), the use of the photolinker allows the isolation of ^{t}Bu-protected peptides, which, in turn, offers the possibility of segment condensations. The N-terminus of hexapeptide **28** was protected as benzyl carbamate and the corresponding ^{t}Bu ester was hydrolyzed. The resulting N-protected peptide and octapeptide **29** were coupled with TBTU/HOBt to give protected *Trichovirin I 1B* (Z-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu).

2. Zusammenfassung

2.1. Einleitung

Peptide, welche α,α-disubstituierte α-Aminosäuren enthalten, sind in ihren konformationellen Freiheiten stark eingeschränkt. Als Folge des starren Peptidrückgrates werden Sekundärstrukturen, wie z. B. β-turns und Helices stabilisiert oder sogar begünstigt. Bei der Suche nach der biologisch aktiven Konformation eines Peptids ist die Herstellung solcher konformationell eingeschränkter Peptide ('*conformationally restricted peptides*') ein wichtiger Ansatz. Peptaibole sind eine Klasse linearer, antibiotisch wirkender Peptide, die einen hohen Anteil von α,α-disubstituierten α-Aminosäuren, vor allem α-Aminoisobuttersäure (Aib), enthalten. Die interessanten biologischen Eigenschaften der Peptaibole werden durch die hochgeordneten Strukturen, welche diese speziellen Peptide ausbilden, erklärt.

Zur Einführung von α,α-disubstituierten α-Aminosäuren in Peptide hat sich die 'Azirin/Oxazolon Methode' als eine äusserst nützliche Methode erwiesen. Dabei werden 2*H*-Azirin-3-amine **1** als Aminosäure-Synthone eingesetzt (*Schema 1*). Die Reaktion von 2*H*-Azirin-3-aminen, wie z.B. dem Aib-Synthon **2**, mit einer Aminosäure oder einer Peptidsäure **3** verläuft in hoher Ausbeute zum Peptid-Amid **4**. Die um eine Aminosäure verlängerte Peptid-Säure **5** wird schliesslich durch die selektive Hydrolyse der terminalen Amid-Gruppe von **4** erhalten. Mit Hilfe der 'Azirin/Oxazolon Methode' konnte in der '*solution-phase chemistry*' eine Vielzahl von sterisch anspruchsvollen α,α-disubstituierten α-Aminosäuren in Peptide eingeführt werden. Mit der Synthese von Peptaibolen und Segmenten davon, hat die 'Azirin/Oxazolon Methode' eine erfolgreiche Anwendung gefunden.



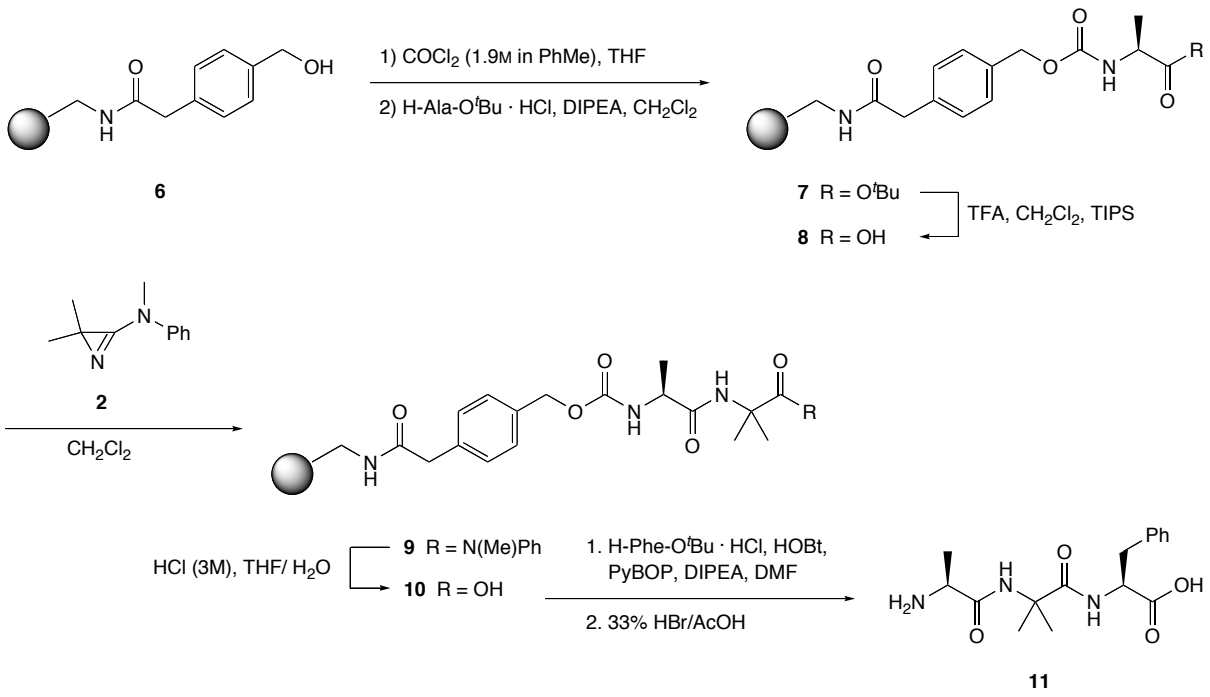
Schema 1

Die Festphasen-Synthese ermöglicht einen schnellen Zugang zu Peptiden ohne die teilweise umständliche Isolation der Peptid-Säure Zwischenprodukte. Das Ziel dieser Dissertation war, nach Möglichkeiten zu suchen, die 'Azirin/Oxazolon Methode' an der Festphase durchzuführen, und damit zusätzlich von deren Eigenschaften zu profitieren.

2.2. Die 'Azirin/Oxazolon Methode' an der Festphase (CHAPTER 4)

Die erste, grundlegende Frage stellte sich bezüglich der Reaktivität: Reagieren denn 2*H*-Azirin-3-amine an der Festphase überhaupt in analoger Weise wie in der Lösung? Um diese Frage zu beantworten, wurde Carboxy-Polystyren-Harz erst mit einer Lösung von 2*H*-Azirin-3-amin **2** und anschliessend, nach ausführlichem Waschen des Harzes, mit einer 3M HCl-Lösung behandelt. Diese beiden Schritte wurden wiederholt. *Attenuated total reflectance* (ATR) FT-IR Messungen zeigten abwechselnd ein Verschwinden und Erscheinen der Carboxy-Absorptions Bande, womit die gestellte Frage positiv beantwortet werden konnte.

In allen Festphasen-Synthesen ist der Linker von entscheidender Bedeutung. Für unsere Verwendung sollte der Linker während der Hydrolyse der terminalen Amidbindung mit 3M HCl und der Entschützung des ^{t}Bu Esters mit Trifluoressigsäure (TFA) stabil, jedoch am Ende der Synthese unter Bedingungen, welche das Peptid nicht beeinträchtigen spaltbar sein. Zur Erfüllung dieser Voraussetzungen hat sich der Carbamat Linker des 'PAM-Resins' als am geeignetsten erwiesen (*Schema 2*).



Schema 2

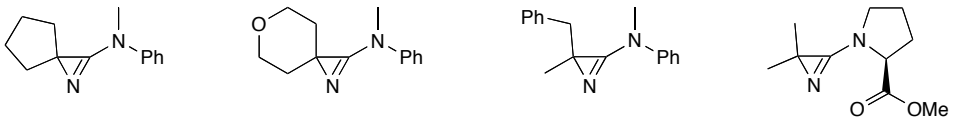
Das Harz **6** wurde mit einer Lösung von Phosgen in Toluol behandelt. Das dabei entstandene Chloroformat-Harz wurde mit H-Ala-O^{t}Bu zu **7** umgesetzt, dessen ^{t}Bu ester mit TFA hydrolysiert wurde. Das Harz **8** wurde anschliessend mit einer Lösung von *N*,2,2-

Trimethyl-*N*-phenyl-2*H*-azirin-3-amin (**2**) zum Peptid-Amid **9** umgesetzt, welches wiederum mit 3M HCl in THF/H_2O zur Peptid-Säure **10** hydrolysiert wurde. Überschüssiges **2** konnte zurückgewonnen und wieder verwendet werden. Zur weiteren Kettenverlängerung wurden entweder ^{t}Bu geschützte Aminosäuren und ein Kupplungsreagenz, wie z. B. PyBOP, oder **2** eingesetzt. Die Abspaltung vom Harz erfolgte mit HBr (33%) in Essigsäure, wobei das Tripeptid **11** erhalten wurde. Mit dieser Methode konnten mehrere Modell-Peptide und einige Peptaibol-Segmente hergestellt werden.

2.3. Einführung Verschiedener α,α-Disubstituierter α-Aminosäuren (CHAPTER 5)

Im nächsten Schritt wurde abgeklärt, ob die an die Festphase angepasste 'Azirin/Oxazolon Methode' auf das Aib-Synthon **2** beschränkt ist, oder ob eine Erweiterung auf andere 2*H*-Azirin-3-amine möglich ist. Es wurde gezeigt, dass die Methode auf das 1-Aminocyclopentan-1-carbonsäure-Synthon **12**, das 4-Aminotetrahydro-2*H*-pyran-4-carbonsäure-Synthon **13** und das α-Methylphenylalanin-Synthon **14** ausgedehnt werden kann (*Abbildung 1*).

Die Synthesen von Peptiden, welche ein poly-Aib Motif enthielten, verliefen aufgrund von Aggregationseffekten nicht erfolgreich.



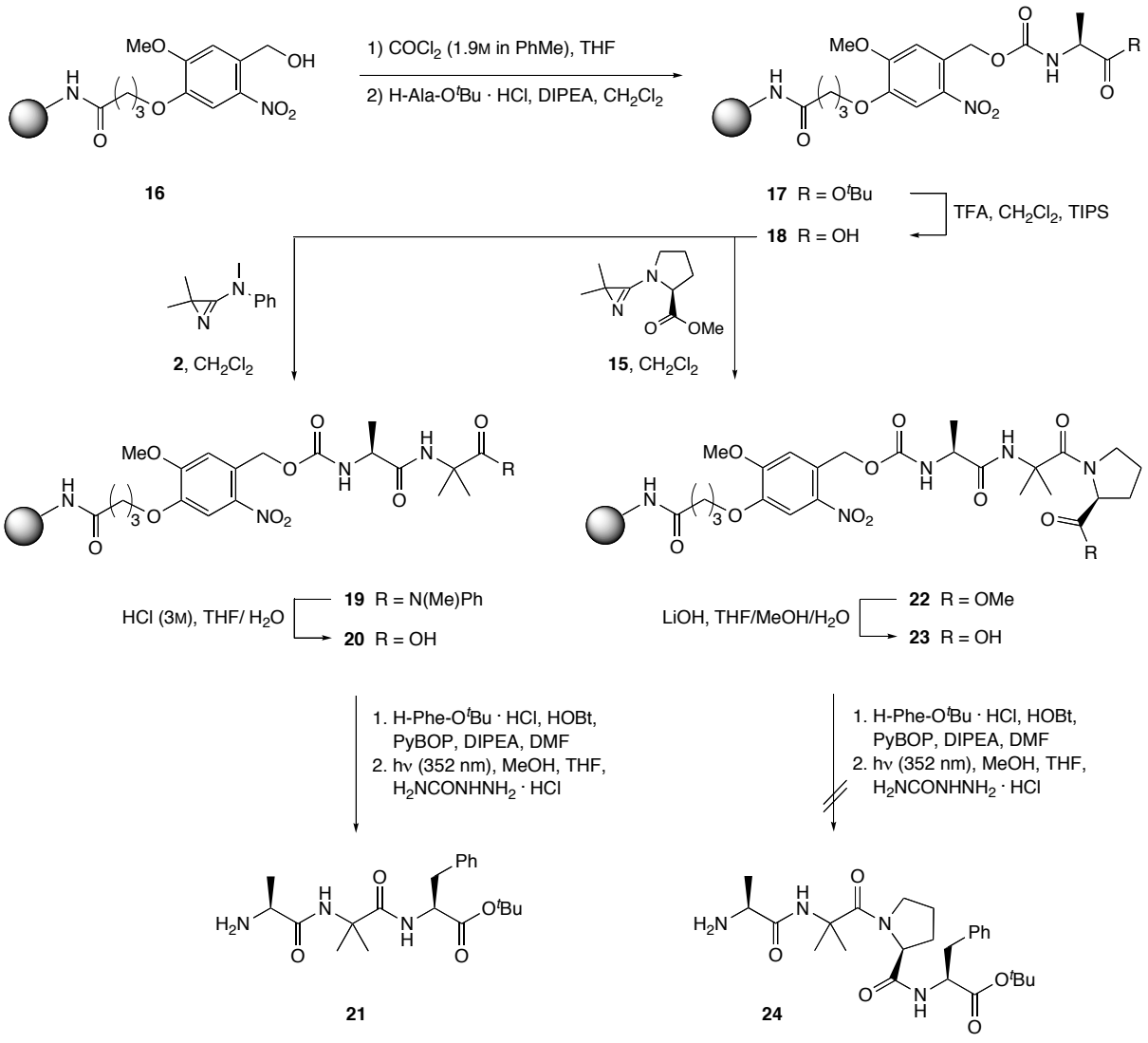
12 **13** **14** **15**

Abbildung 1

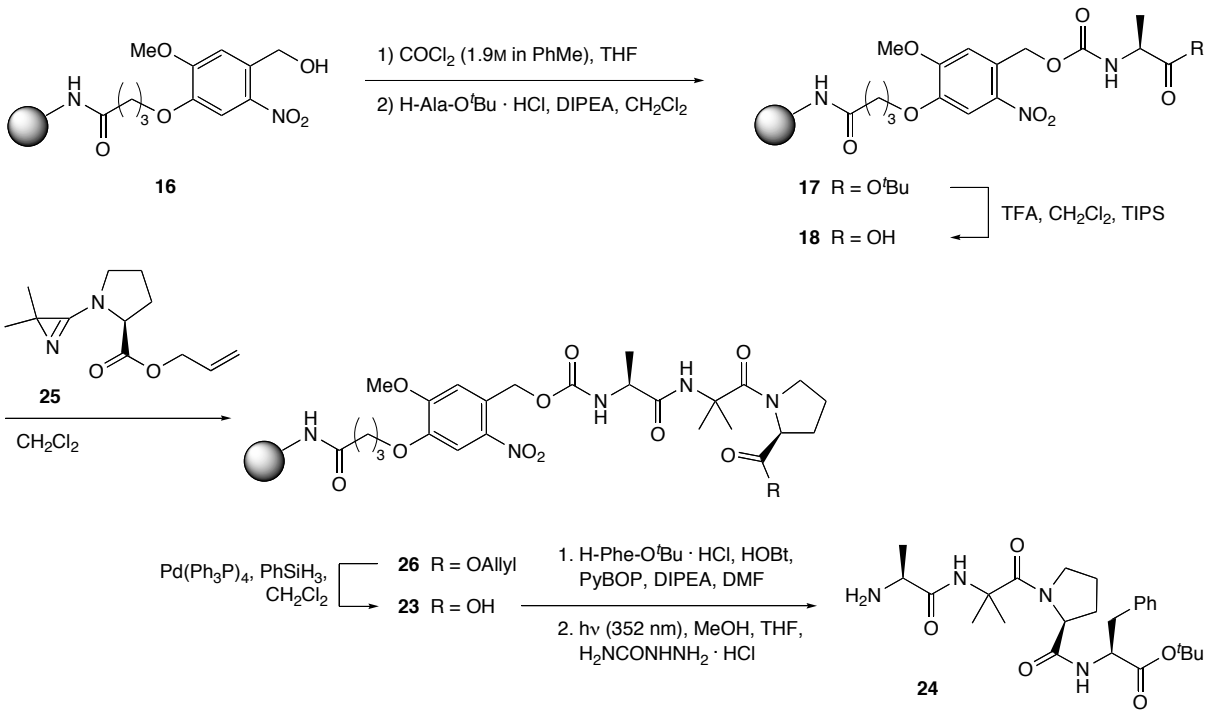
2.4. Einführung der Aib-Pro Einheit (CHAPTERS 6 AND 7)

Da der grösste Teil der Peptaibole eine Aib-Pro Sequenz enthält, war es von grossem Interesse, diese Einheit mit einem Dipeptid-Synthon einzuführen. Als jedoch das Dipeptid-Synthon **15** (*Abbildung 1*) eingesetzt wurde, scheiterte das Experiment an der Empfindlichkeit der Aib-Pro Amidbindung gegenüber starken Säuren wie HBr, die zur Spaltung des Linkers eingesetzt wurde.

Während ein Pd^0-labiler Linker nicht zu den gewünschten Resultaten führte, gelang mit dem photolabilen Nitroveratryl-Linker **16** die Synthese des Modell-Peptides H-Ala-Aib-Phe-O^{t}Bu (**21**) (*Schema 3*). Als jedoch zur Einführung der Aib-Pro Einheit das Dipeptid-Synthon **15** verwendet wurde, scheiterte das Experiment erneut (*Schema 3*). Während der Verseifung von **22**, wurde eine deutliche Verdunkelung des Harzes beobachtet, was wiederum die Photolyse des Linkers verunmöglichte. Um dieses Problem zu lösen, wurde versucht 2*H*-Azirin-3-amine vom Typ **15** mit leicht entfernbaren Carboxy-Schutzgruppen herzustellen. Schliesslich gelangen die Synthesen des Phenacyl-Ester und des Allyl-Ester (**25**) geschützten Dipeptid-Synthons. Letzteres konnte erfolgreich zur Synthese von H-Ala-Aib-Pro-Phe-O^{t}Bu (**24**) benutzt werden, indem der oben beschriebene Photolinker und ein auf Pd^0 basierendes Verfahren zur Entschützung des Allyl-Esters **26** verwendet wurden (*Schema 4*).



Schema 3



Schema 4

2.5. Anwendungen (CHAPTER 7)

Zur Evaluation der 'Azirin/Oxazolon Methode' an der Festphase, wurde versucht, Aib-Pro enthaltende Peptaibol-Segmente zu synthetisieren. H-Val-Aib-Gly-Aib-Aib-Pro-Leu-O^{t}Bu (**27**; A8 – A14 von *Trichovirin Ia*), H-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-O^{t}Bu (**28**; A1 – A6 von *Trichovirin I 1B*) und H-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu (**29**; A7 – A14 von *Trichovirin I 1B*) wurden mit Hilfe des Photolinkers **16**, dem Aib-Synthon **2** und dem Aib-Pro-Synthon **25** hergestellt.

Im Gegensatz zur 'PAM/HBr'-Strategie (*Schema 2*), erlaubt die Verwendung des Photolinkers die Herstellung von ^{t}Bu-geschützten Peptiden, welche wiederum die Möglichkeit zu Segmentkondensationen eröffnet. Der N-Terminus des Hexapeptides **28** wurde als Benzyl Carbamat geschützt und der ^{t}Bu Ester des entstandenen Peptids hydrolysiert. Das N-geschützte Hexapeptid konnte erfolgreich mit dem Octapeptid **29** und TBTU/HOBt als Kupplungsreagenz zu geschütztem *Trichovirin I 1B* (Z-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu) gekoppelt werden.

3. General Introduction

3.1. Properties and Structure of Peptides and Proteins

3.1.1. General

Proteins are one of the classes of biopolymers, along with nucleic acids, polysaccharides, and lipids, that make up the fundamental constituents of living cells. The name 'protein' is derived from the Greek 'πρωτειοσ' (proteios), meaning 'of primary importance'.

Proteins are essential to the structure and function of a cell. As enzymes they are able to catalyze complex biochemical reactions. They serve as transport and storage molecules (hemoglobin, ferritin), as molecular motors (myosin, kinesin, dynein), as biological scaffolds for the mechanical integrity and tissue (keratin, collagens), as hormones (follitropin (FSH), thyrotropin), as receptors for hormones and neurotransmitters, as regulators (enzyme inhibitors, transcription factors), and are present in the immune system (immunglobulins, complement). Furthermore, they play an important role in vision (rhodopsin), and in the exceedingly compact organization of the DNA (histones). Besides carbohydrates and lipids, they act as the third large group of nutrition and storage compounds.

Peptides and proteins are assembled from 20 different L-α-amino acids, which are linked by peptide bonds. Alongside these 20 proteinogenic amino acids, others (*e.g.*, α-aminoisobutyric acid, *vide infra*) are rare in nature. Peptides are oligomers, while proteins are built from 100 and more (up to many thousands) amino acids [1].

3.1.2. The Structure of Peptides and Proteins

Proteins are folded into unique three-dimensional structures. The sequence of the amino acids determines the shape into which the protein naturally folds (native state). The structure of a protein can be assigned to its primary-, secondary-, tertiary-, and quaternary-structure. The primary structure refers to the amino acid sequence of the polypeptide chain of a protein, while the secondary structure is the locally defined arrangement of the polypeptide chain. These segments are highly organized elements such as α-helices, β-sheets, and turns. The folding of the whole polypeptide chain determines the overall shape of a single protein molecule and is known as the tertiary structure. Finally, the assembly of several polypeptide chains, called protein subunits, results in the overall shape of the protein, and is defined as the quaternary structure of a protein [1].

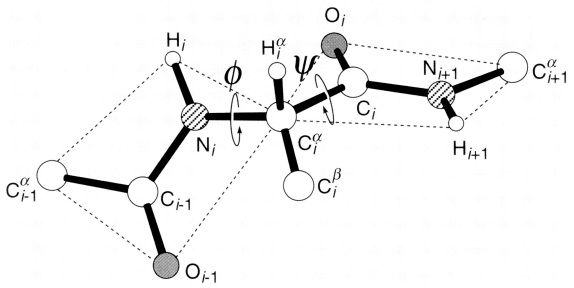


Figure 1. *The Torsion Angles* Φ *and* Ψ *of the Peptide Backbone.*

Due to mesomeric stabilization, the amide bond is nearly planar and usually prefers a *s-trans*-configuration. In order to describe the local shape of the amino acids, and therefore to classify secondary structures, the torsion angels Φ and Ψ were introduced (*Figure 1*). Φ is defined as the torsion angle of the N–C(α) bond, and Ψ as the torsion angle of the C(α)–CO bond, respectively. Viewing from the C(α) atom, clockwise rotation results in a plus sign (*et vice versa*). A *Ramachandran*-Plot (or diagram) is the plot of Φ against Ψ and shows favorable combinations of Φ and Ψ, which result from minimizing *Pitzer-*, *Newman-*, and 1,3-allylic-strain, as well as from an conformation that enables optimal H-bonding (*Figure 2*) [2]. The more restricted the conformational freedom of an amino acid is, the smaller is the area of favored (or 'allowed') conformations in the *Ramachandran*-Plot (chapter 3.1.4).

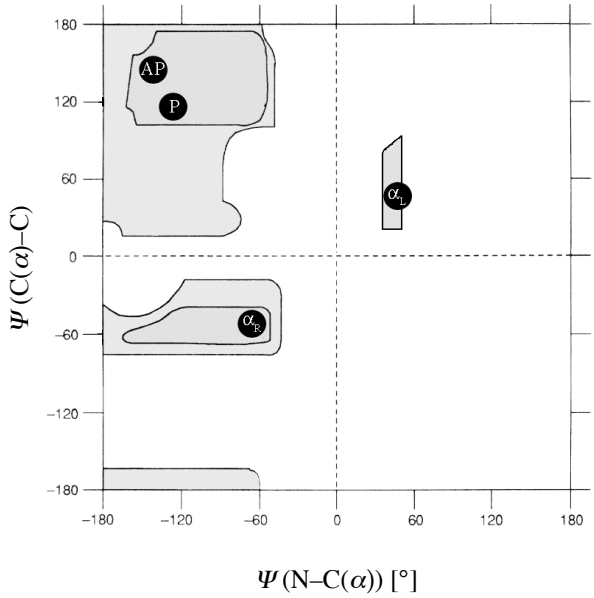


Figure 2. Ramachandran-*Plot for Poly-*L*-Alanine* (α_R/α_L*: Right/Left Handed* α*-Helix; P: Parallel* β*-Sheet; AP: Antiparallel* β*-Sheet).*

The most important secondary structures are the α- and the 3_{10}-helix, the parallel and the antiparallel β-sheet, as well as β- and γ-turns (*Table 1*) [3–5].

Table 1. *Torsion Angles* Φ *and* Ψ *of Common Secondary Structures.*

Structure	Φ^{i+1} [°]	Ψ^{i+1} [°]	Φ^{i+2} [°]	Ψ^{i+2} [°]
α-helix (right/left handed)	–(+)58	–(+)47	–(+)58	–(+)47
3_{10}-helix (right/left handed)	–(+)60	–(+)30	–(+)60	–(+)30
parallel β-sheet	–119	+113	–119	+113
antiparallel β-sheet	–139	+135	–139	+135
β-turn type I and I'	–(+)60	–(+)30	–(+)90	0
β-turn type II and II'	–(+)60	+(–)120	+(–)80	0
β-turn type III and III'	–(+)60	–(+)30	–(+)60	–(+)30
γ-turn	+70 to +85	–60 to –70		
inverse γ-turn	–70 to –85	+60 to +70		

3.1.3. Peptide Design

Often, only a small peptide segment gives rise to the biological activity of a protein. When searching for peptide mimetics, this segment is determined and its biologically active conformation has to be found. A basic approach when searching for the biologically active conformation of a peptide segment is the synthesis of conformationally restricted peptides [6–9]. Furthermore, medicinal chemists commonly also turn to unnatural analogues in search of metabolic stability.

By introducing *local constraints*, modifications are performed, which stabilize or even favor secondary structures such as α-helices or β-turns. The most commonly used method to restrict the conformational freedom of a peptide is the alkylation of the amide N-atom or the C(α)-atom of the peptide backbone, or the formation of cyclic structures. In *N*-alkylated amino acids, the ability of the NH-group for H-bonding is lost, and a bulky alkyl group is inserted into the backbone. Consequently, *N*-alkylated amino acids are strong helix-disrupters [10]. In contrast to primary amides, the preference for *trans*-amide bonds is strongly reduced in *N*-alkylated amino acids. In fact, the *cis*- and *trans*-amide conformations are nearly isoenthalpic, and *N*-methyl amino acids often adopt, like proline, *cis*- as well as *trans*-amide conformations in peptides. Further characteristics of *N*-alkylated amino acids are their

enhanced hydrophobicity and an improved stability towards proteolytic degradation increasing their bioavailability and therapeutical potential [6 and refs. cited therein, 11]. Since C(α)-alkylated (α,α-disubstituted α-amino acids) are of great importance, they are discussed in chapter 3.1.4. Further possibilities to modify the peptide chain are the introduction of β-amino acids [12–14], the introduction of unnatural D-amino acids [6, 15], or the exchange of the amide group by one of the following groups: CO–O (depsipeptides) [16], CS–NH (endothiopeptides) [17], NH–CO (retropeptides) [18], CH_2–NH (reduced) [19], CH_2–S [20], CH_2–SO [20], CO–CH_2 [21], CH=CH [22], and CH_2–CH_2 [23].

In order to introduce *global constraints*, covalent bonds between distant parts in the sequence are formed. This affects drastically the overall conformation of the peptide as well as segments within the formed cycle adopt more defined conformations than in the linear form. The formation of lactames, lactones, (thio)ethers, and disulfidebridges are the most common possibilities to achieve cyclization, and it can be performed by linking the backbone termini or by using functional groups of the side chains.

3.1.4. α,α-Disubstituted α-Amino Acids in Peptides

The fourfold substitution of the C(α)-atom causes a drastic restriction of the conformational freedom of an amino acid. In the corresponding *Ramachandran*-Plots, 50% of the area are accessible to glycine, 16% to alanine, while only a few percent remains accessible to α-aminoisobutyric acid (Aib) (*Figure 3*). The region allowed for the Aib-residue includes both the left- and right-handed α- and 3_{10}-helices. Thus, the introduction of Aib or other α,α-disubstituted α-amino acids into peptides is particularly useful, since it promotes or favors turns or helical conformations [6, 24].

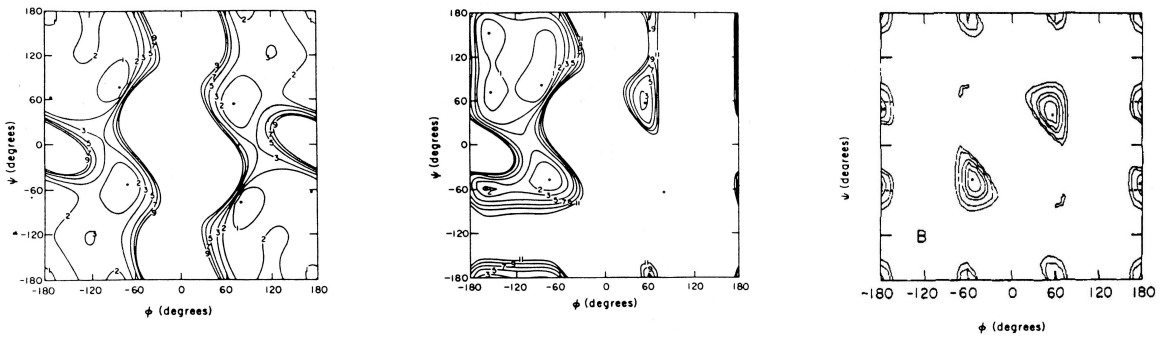


Figure 3. Ramachandran-*Plots for Gly (A),* L-*Ala (B), and Aib (C).*

Examples of α,α-disubstituted α-amino acids are the strongly helix-inducing, and probably best examined representative, α-aminoisobutyric acid (Aib), the most simple chiral α,α-disubstituted α-amino acid isovaline (Iva), 1-aminocyclpentanecarboxylic acid (Acp) which behaves, in terms of conformational characteristics, very similar to Aib, and α-methylphenylalanine (Phe(2Me)) (*Figure 4*).

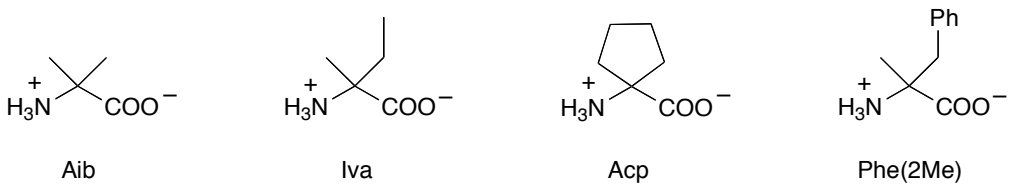


Figure 4. *Examples of* α,α*-Disubstituted* α*-Amino Acids.*

A class of α,α-disubstituted α-amino acids containing peptides, the peptaibols, have attracted great interest, due to their biological activities. Peptaibols are linear, amphiphilic oligo*pept*ides from fungal sources with a high proportion of α,α-disubstituted α-amino acids, above all, *Aib*, and containing a C-terminal alcoh*ol* [25, 26]. Peptaibols show antibiotic properties due to self association in lipid membranes forming ion channels [27]. Prominent representatives among the so far 309 known different peptaibol sequences are *Alamethicin*, *Antiamöbin*, *Hypomurocin, Trichovirin*, and *Zervamicin* sequences [28].

3.2. Peptide Synthesis

3.2.1. The Formation of the Peptide Bond

Since carboxylic acids and amines form at moderate temperatures only salts, an activation of the carboxyl- or amino-component is necessary to form a peptide bond. Most often, the activation of the carboxyl-component is realized by turning the OH group into a good leaving group. The reactive acylating agent can be prepared in a separate step in advance to the nucleophilic attack of the amino-component, or in presence of it by using coupling reagents.

The probably most popular coupling method is the use of coupling reagents. The first member of this class, *N*,*N*'-dicyclohexylcarbodiimide (DCC), was introduced by *Sheehan* in 1955 (*Figure 5*) [29]. DCC remains one of the most popular and widespread coupling reagents. Its principal limitation is the dehydration of asparagine and glutamine residues, although this can largely be prevented by adding 1-hydroxy-1*H*-benzotriazole (HOBt). In solid-phase peptide synthesis the principal drawback to the use of DCC is the formation of dicyclohexylurea which is insoluble in CH_2Cl_2. To overcome this issue, other carbodiimides such as *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide (EDC) [30], *N*,*N'*-diisopropylcarbodiimide [31, 32], *N*-*tert*-butyl-*N'*-methylcarbodiimide [33, 34], *N*-*tert*-butyl-*N'*-ethylcarbodiimide [33] were developed, which form ureas being more soluble in CH_2Cl_2 than dicyclohexylurea.

Other widely used coupling reagents are based on phosphonium or amidinium (or uronium) salts which, in the presence of a tertiary base (*e.g.*, ethyldiisopropylamine, *N*-methylmorpholine, collidine), smoothly convert protected amino acids to a variety of activated species. The most commonly employed, (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) [35], (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) [36], 2-(3-oxido-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylamidinium hexafluorophosphate (HBTU) [37], 2-(3-oxido-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylamidinium tetrafluoroborate (TBTU) [37], generate OBt esters, which have found wide application in solution- and solid-phase peptide synthesis (*Figure 5*). BOP can be substituted by PyBOP without loss of performance. The substitution of BOP is highly recommended since when using it, the highly carcinogenic by-product HMPA is formed. Other coupling reagents such as 2-(3-oxido-7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylamidinium hexafluorophosphate (HATU) [38, 39] or 2-(6-chloro-3-oxido-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylamidinium hexafluorophosphate (HCTU) [40] generate esters that are more reactive than OBt esters (*Figure 5*). When using coupling

reagents for peptide bond formation, HOBt or 1-hydroxy-7-aza-1*H*-benzotriazole (HOAt), respectively, are added to improve performance and to reduce epimerization/racemization.

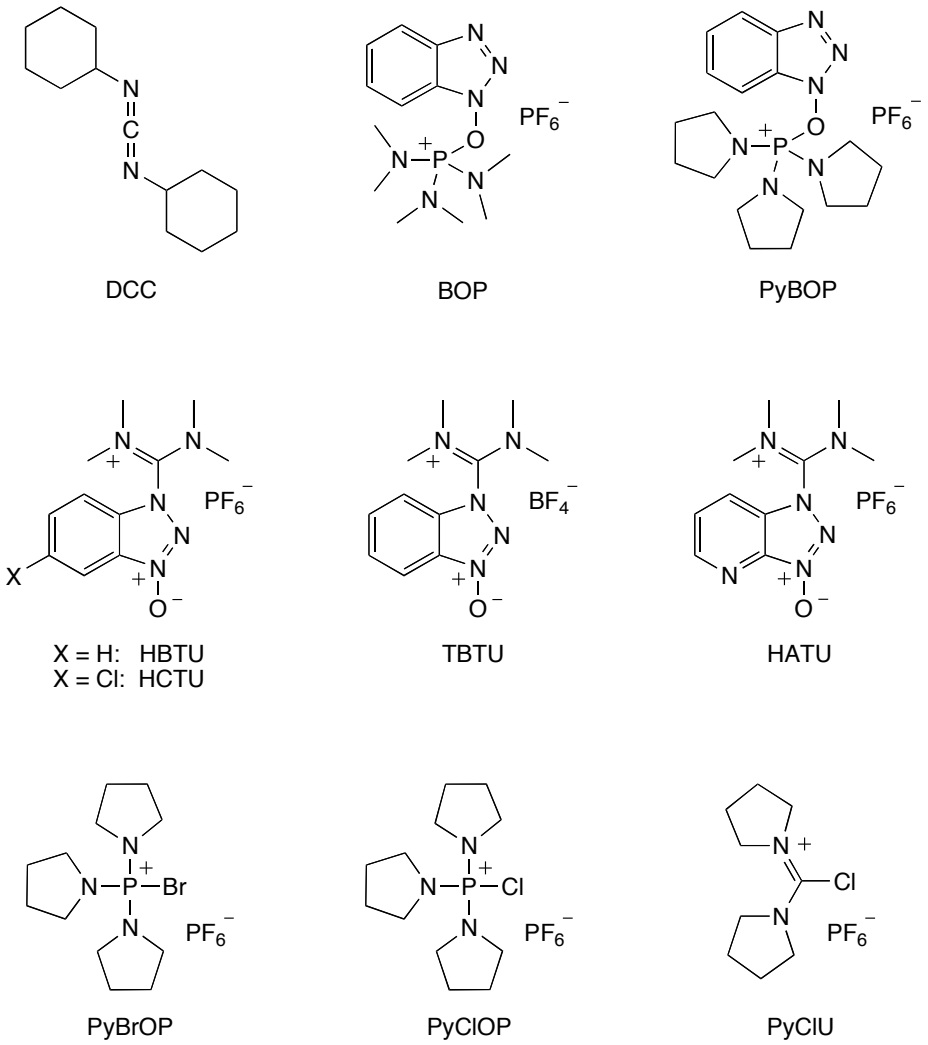
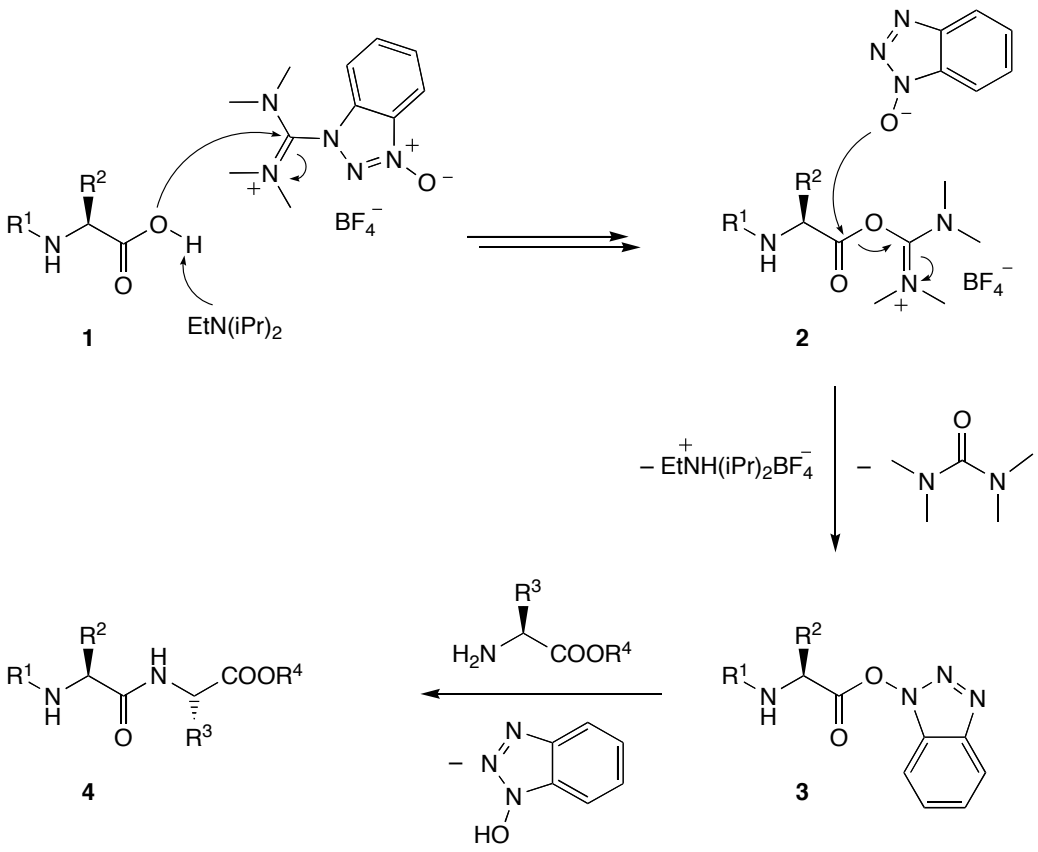


Figure 5. *Common Coupling Reagents*.

The formation of the peptide bond using TBTU as the coupling reagent is outlined in *Scheme 1*. The base, most often a sterically demanding tertiary amine such as $EtN(iPr)_2$, deprotonates the N-protected amino acid or the N-protected peptide acid (**1**). Then, the resulting carboxylate anion attacks the electrophilic amidinium C-atom of TBTU and $^-$OBt is expelled. After a nucleophilic attack of $^-$OBt and elimination of tetramethylurea, the activated OBt ester **3** is formed. The attack of the C-protected amino acid or the C-protected peptide affords the corresponding peptide **4** by expelling HOBt.



Scheme 1. *TBTU as the Coupling Reagent for Peptide Bond Formation.*

For the coupling of *N*-methyl amino acids, the use of PyBrOP, PyClOP, or PyClU provides a significant improvement over the HOBt-based reagents (*Figure 5*) [41, 42]. For the introduction of the sterically demanding α,α-disubstituted α-amino acids, coupling reagents were applied with various levels of success [42, 43]. In general, the introduction of α,α-disubstituted α-amino acids into peptides is complicated by inefficient coupling, racemization, and cyclization to give diketopiperazines (attributed to the gem. dialkyl effect) [42]. The 'azirine/oxazolone method' (chapter 3.2.2) and the use of N-protected amino acid fluorides (*vide infra*) have proven to be valuable protocols for the introduction of the bulky α,α-disubstituted α-amino acids.

Preformed active esters such as pentafluorophenyl esters [44–46], urethane-protected amino acid *N*-carboxyanhydrides (UNCA's) [47], and preformed symmetrical anhydrides (PSA) [48, 49] are also efficient acylating agents. PSA have been used in many research groups, mainly in Boc solid-phase peptide chemistry, because of their high reactivity. They are generated using two equivalents of protected amino acid and one equivalent of DCC in

CH_2Cl_2. Some N-protected amino acids are not soluble in CH_2Cl_2 and require significant amounts of *N*,*N*-dimethylformamide (DMF) for solubilization, but DMF slows down the rate of the formation of the activated derivative. Another drawback is that the preparation and use of PSA is quite wasteful, since two equivalents of the amino acid are required to form one equivalent of the activated species.

Although known since the beginning of the last century [50], N-protected amino acid chlorides have been rarely used in peptide synthesis. Several decades later, [(9*H*-fluoren-9-yl)methyloxy]carbonyl (Fmoc) amino acid chlorides were successfully applied to synthesize short peptide segments [51–55]. Due to their high reactivity and sensitivity to hydrolysis, the amino acid chlorides have to be prepared immediately before use. Moreover, in amino acid chlorides the carboxy group is somewhat 'overactivated', which may lead to racemization/epimerization and to the formation of a multitude of side products. Another drawback is the impossibility of obtaining stable Fmoc amino acid chlorides from trifunctional amino acids bearing side chain protection incorporating the *tert*-butyl moiety.

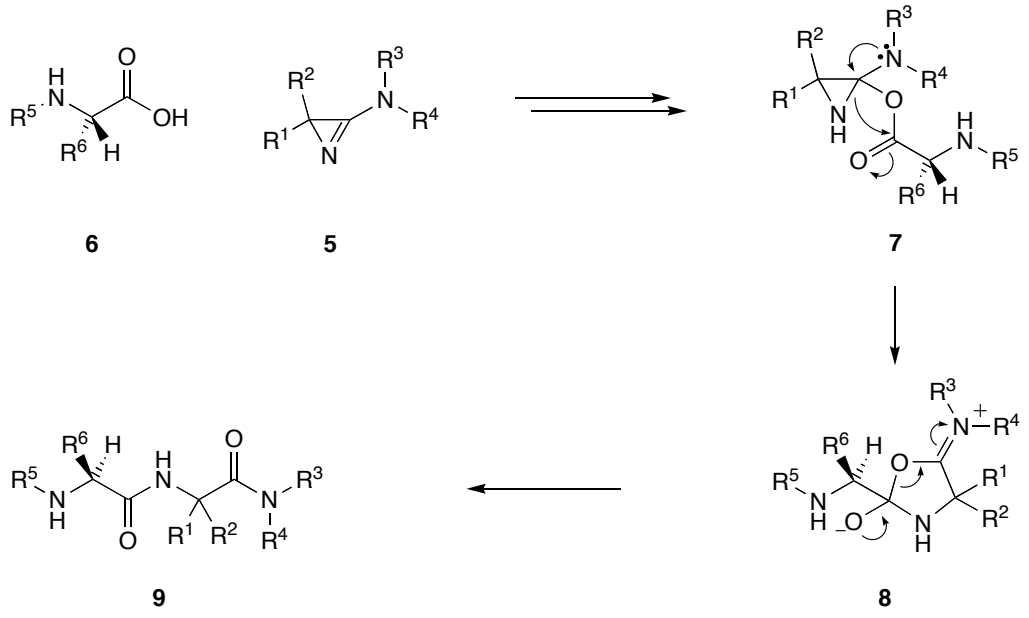
On the other hand, amino acid fluorides are of greater stability than the corresponding chlorides, but appear to be of nearly equal reactivity toward amines. N-protected amino acid fluorides can be prepared by the reaction of the corresponding N-protected amino acids with cyanuryl fluoride, and have been used successfully in solution- and solid-phase peptide synthesis [56–60]. In regarding the incorporation of the bulky α,α-disubstituted α-amino acid Aib into peptides, the use of Fmoc-protected amino acid fluorides has been shown to be superior in comparison with PSA's, UNCA's, and PyBroP activation.

3.2.2. The 'Azirine/Oxazolone Method'

As already mentioned in the previous chapter, the introduction of α,α-disubstituted α-amino acids into peptides is complicated by inefficient coupling, racemization/epimerization, and diketopiperazine formation when using coupling reagents.

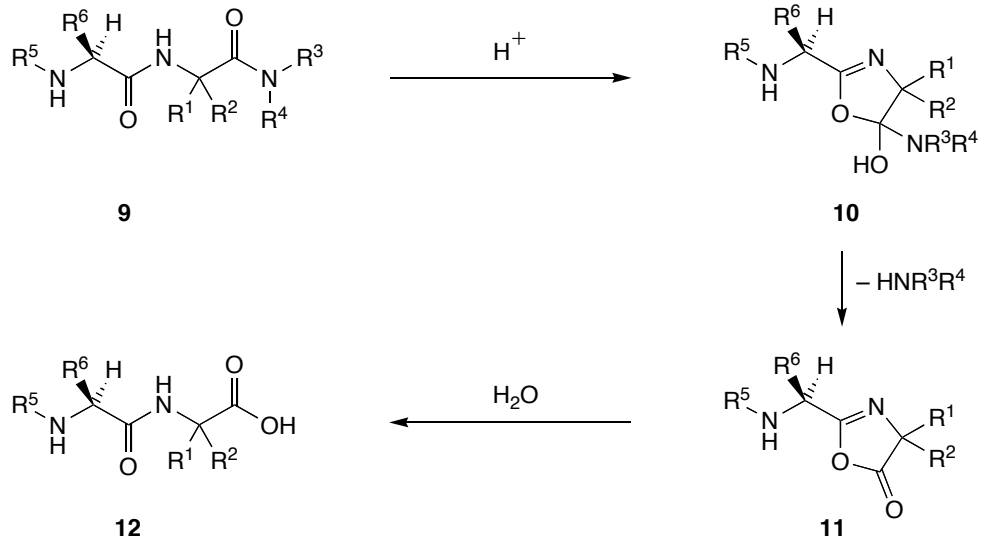
One useful, and very efficient method for the introduction of the sterically demanding α,α-disubstituted α-amino acids is the 'azirine/oxazolone method' [61–63]. Thus, the reaction of 2*H*-azirin-3-amines **5**, which represent the amino acid synthons, with amino or peptide acids leads to peptide amides, the terminal amide bonds of which can be hydrolyzed selectively to give the extended peptide acids (*Schemes 2* and *3*). Due to considerable ring strain, the 2*H*-azirin-3-amine represents the activated amino component. Therefore, an activation of the carboxyl component is no longer necessary, and no further reagents are required, which avoids, in turn, the formation of side products, and finally facilitates the work up.

In a first step, the protected amino acid or peptide acid **6** protonates the 2*H*-azirin-3-amine **5**, which is then attacked by the carboxylate anion at its C(3)-atom. A ring enlargement of aziridine **7** leads to the zwitterion **8**, which provides the extended peptide amide **9** in a ring opening reaction.



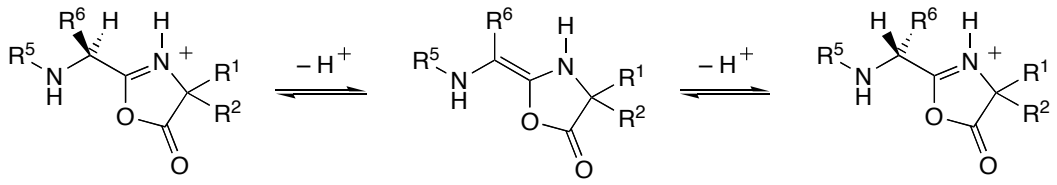
Scheme 2. *Mechanism of the 'Azirine/Oxazolone Method'. Reaction of the* 2H*-Azirin-3-amine with an Amino or Peptide Acid.*

Under acidic conditions (3M HCl, H_2O/THF), peptide amide **9** can be hydrolyzed *via* the formation of an 1,3-oxazol-5(4*H*)-on intermediate **11** to give the peptide acid **12**. In comparison with *N*,*N*-dimethylamides, the hydrolysis of *N*-methylanilides (R^3 = Me, R^4 = Ph) is faster, and therefore, the danger for racemization of the penultimate amino acid is smaller (*Scheme 4*).



Scheme 3. *Mechanism of the 'Azirine/Oxazolone Method'. Hydrolysis of the Terminal Amide Bond.*

The 'azirine/oxazolone method' has proven to be successful for the introduction of a variety of sterically demanding α,α-disubstituted α-amino acids into oligopeptides [64–73], endothiopeptides [74–76], cyclic peptides [77–78], and cyclic depsipeptides [79–82]. Moreover, the 'azirine/oxazolone method' has found application in the synthesis of several peptaibols, or segments thereof [64, 68–73].



Scheme 4. *Racemization of the Penultimate Amino Acid.*

3.3. 2*H*-Azirin-3-amines

3.3.1. Synthesis and Properties of 2*H*-Azirin-3-amines

2*H*-Azirin-3-amines **5** are three-membered, cyclic amidines with an endocyclic CN-double bond, and are derivatives of the 2*H*-azirines **13** (*Figure 6*). The structure of several 2*H*-azirin-3-amines have been determined by X-ray crystal-structure determination. The structures show a short C(2)–C(3)- and a long N(1)–C(2)-single bond, alongside with a lengthened N(1)–C(3)-double bond and a shortened C(3)–N(4)-single bond in common, indicating delocalization of the N(4) lone-pair with the π-system of the CN-double bond [61, and Refs. cited therein].

5 **13**

Figure 6. *2*H-*Azirin-3-amines and 2*H-*Azirines*.

The disubstituted N(1)-atom is, as usual in amidines, the most nucleophilic and most probably also the most basic atom. In comparison with acyclic amidines, the basicity of the 2*H*-azirin-3-amines is lower, due to a different hybridization of the N-atom. While the acyclic system allows a sp^2-hybridization of the N-atom with interorbital-angles of 120°, a non-hybridizied N-atom is favored in three-membered rings (interorbital angles of 90°). Consequently, the lone-pair is located in a *s*-orbital, and therefore, less basic (*Figure 7*) [61].

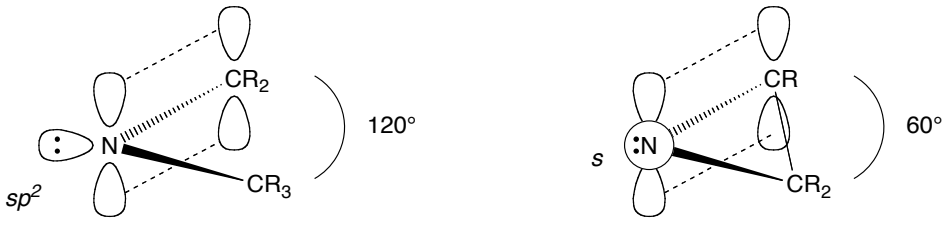
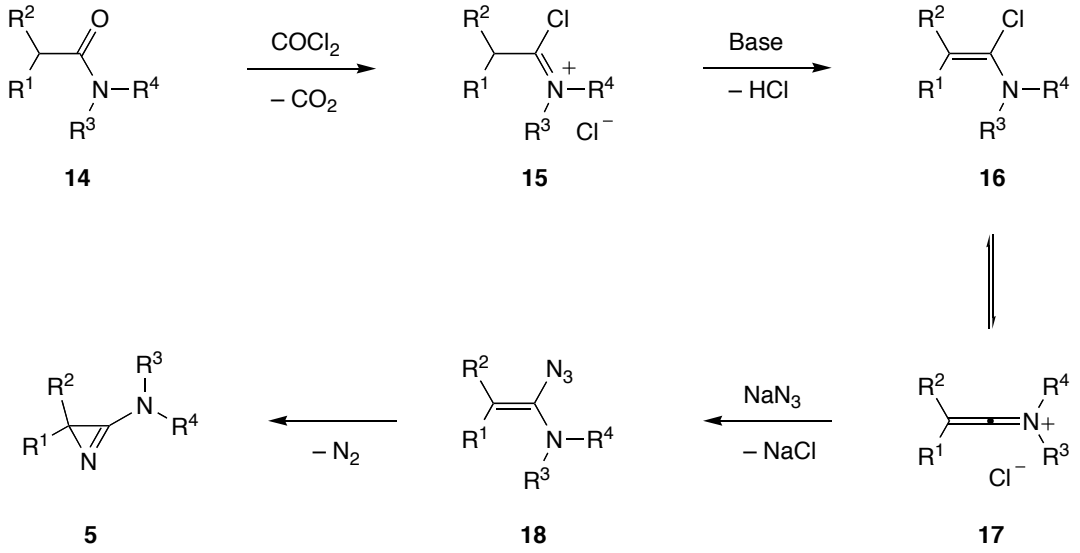


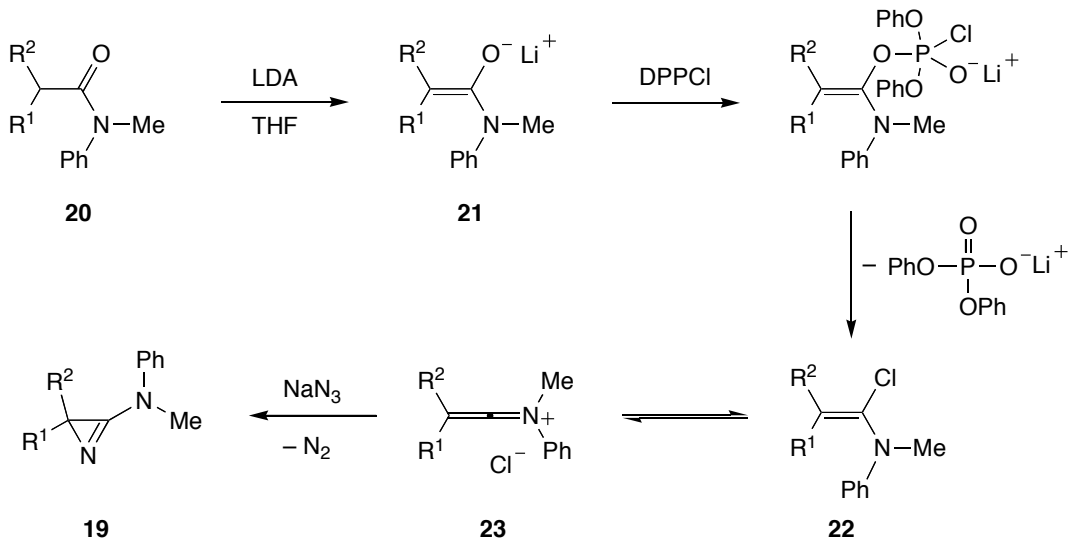
Figure 7. *Hybridization of the N-Atom in a 3-Membered Ring and in an Acyclic Structure*.

The first syntheses of 2*H*-azirin-3-amines were carried out by *Rens* and *Ghosez* in 1970 [83]. *N*,*N*-Disubstituted amides **14**, containing at least one α-H-atom, were treated with phosgene to give chloroiminium chlorides **15**, which, after base-promoted HCl-abstraction, afforded the α-chloroenamins **16** (*Scheme 5*). By addition of NaN_3, the α-chloroenamines **16** react *via* their corresponding keteniminiumsalts **17** to α-azidoenamines **18**, which, in turn, afford under N_2-loss the 2*H*-azirin-3-amines **5**. For less reactive amides (R^1 and/or R^3 = aryl) modified procedures are used. Thus, the addition of catalytic amounts of *N*,*N*-dimethylformamide during the reaction of the amide and phosgene leads to a spontaneous formation of the α-chloroenamine [62]. Another modification is proceeding *via* the corresponding thioamides [84], which are, in general, easily accessible by thionation of the amides with *Lawesson*-reagent [85, 86].



Scheme 5. *Synthesis of* 2H-*Azirin-3-amines According to* Rens *and* Ghosez.

N-Methyl-*N*-phenyl-2*H*-azirin-3-amines **19** can alternatively be obtained by the method of *Villalgordo* and *Heimgartner* which avoids the use of toxic phosgene [87, 88]. Thus, amide **20** is deprotonated with LDA and the resulting amide enolate **21** is converted into the α-chloroenamine **22** by treatment with diphenylphosphorylchloride (DPPCl). In turn, **22** reacts analogously to the method of *Rens* and *Ghosez* with NaN_3 to give the *N*-methyl-*N*-phenyl-2*H*-azirin-3-amine **19** (*Scheme 6*).



Scheme 6. *Synthesis of* N-*Methyl*-N-*phenyl*-2H-*azirin-3-amines According to* Villalgordo *and* Heimgartner.

Optically active C(2)-monosubstituted 2*H*-azirin-3-amines are obtained *via* a modified *Neber*-rearrangement from the corresponding amino acid [89]. Other access to 2*H*-azirin-3-amines is reported in the literature [90–92].

3.3.2. Reactions of 2*H*-azirin-3-amines

The versatile reactivity of 2*H*-azirin-3-amines **5** is caused by the nucleophilic amidine function, which is part of the highly strained azirine structure (200 KJ/mol). Depending on the reaction conditions, each of the three ring bonds can be opened selectively, leading to reactive intermediates (*Scheme 7*) [61].

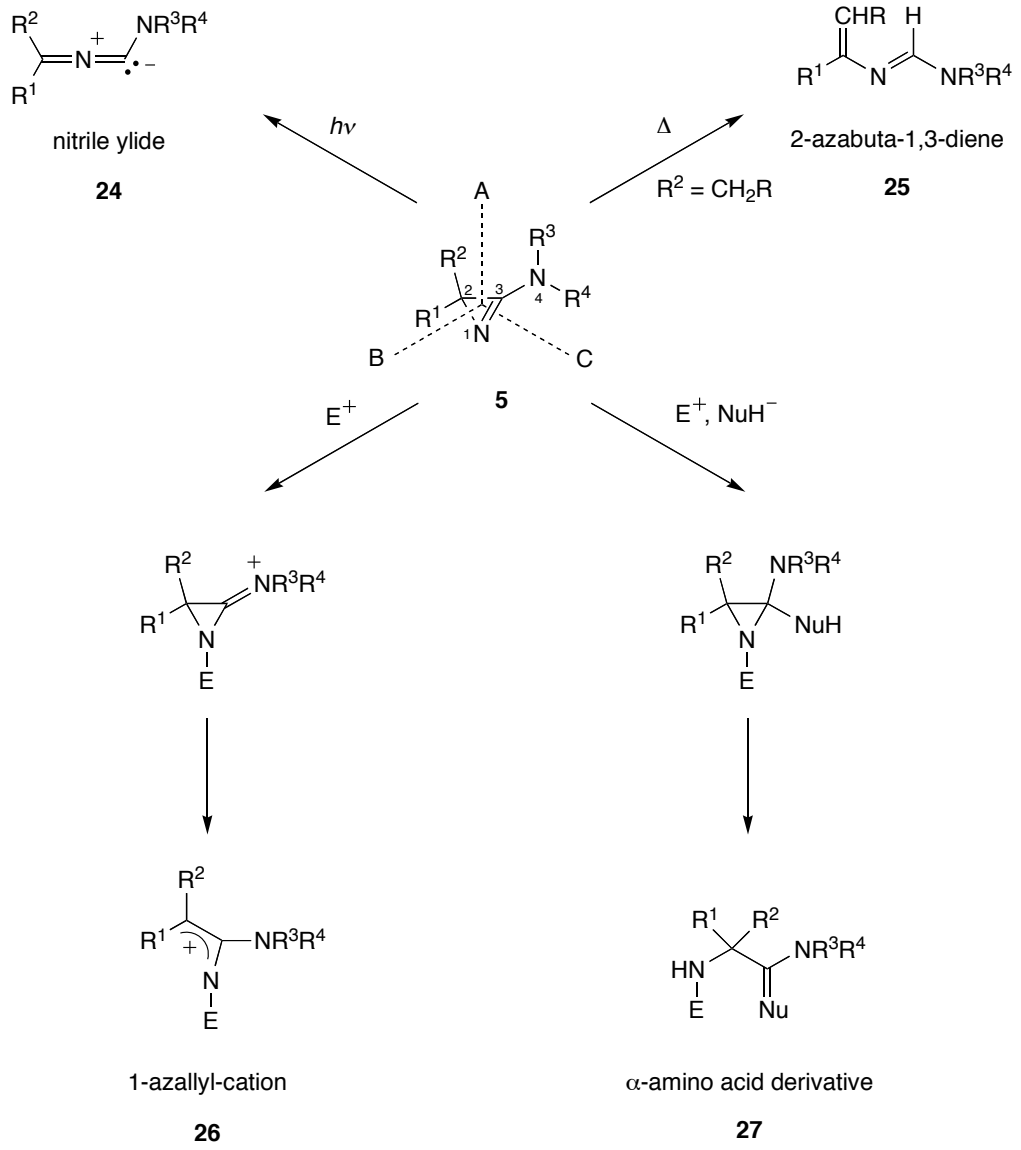
A: *Cleavage of the C(2)–C(3)-single bond*. Irradiation leads to nitrile ylides **24**. If the irradiation is performed in the presence of a dipolarophil, five-membered heterocycles are formed. Pyrolysis at 300–400°C results in the formation of 2-azabuta-1,3-dienes **25**, which can be used in *Diels-Alder* reactions for the synthesis of pyridines.

B: *Cleavage of the N(1)–C(2)-single bond*. In the presence of strong proton-acids and under non-nucleophilic conditions, reactive 1-azallyl-cations **26** are formed.

C: *Cleavage of the N(1)–C(3)-double bond*. 2*H*-Azirin-3-amines **5** react with carboxylic acids, thiocarboxylic acids, sulfinic acids, activated phenols and thiophenols, and with cyclic,

enolizable 1,3-diketones forming amino acid derivatives **27**. In general, the reactions proceed smoothly and already at 0°C. This reaction path starts with the protonation of the 2*H*-azirin-3-amine **5**, and therefore a $pk_a < 8$ of the substrate is required.

For Peptide synthesis, path C is of interest, which was already discussed in more detail (chapter 3.2.2).



Scheme 7. *General Reactions of* 2H-*Azirin-3-amines*.

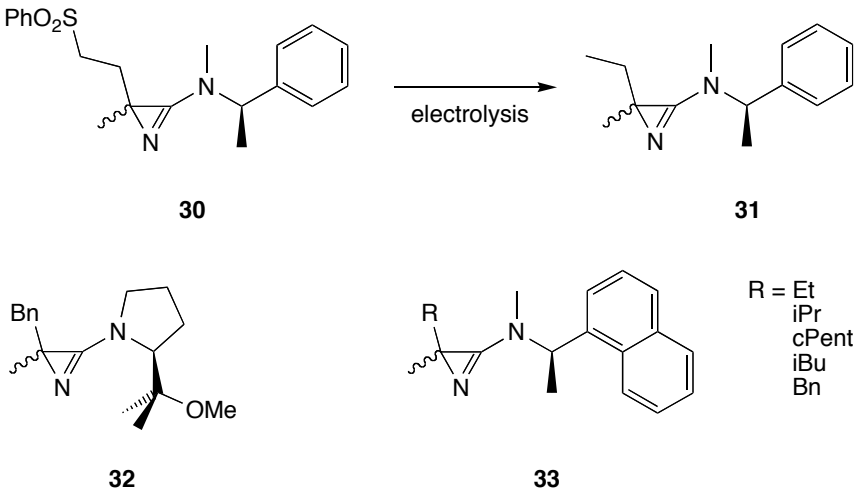
3.3.3. Optically Pure 2*H*-Azirin-3-amines

For peptide synthesis by means of the 'azirine/oxazolone method', optically pure 2*H*-azirin-3-amines were desired. Since the above described syntheses only led to racemic mixtures when different substituents were present at C(2), chiral auxiliaries were introduced with the aim of obtaining separable diastereoisomers. Although no selectivity in the syntheses was achieved, the diastereoisomeric mixtures of **28** and **29** could be separated by means of preparative HPLC (*Figure 8*) [93, 94].

28 **29**

Figure 8. *The First Separable Mixtures of Diastereoisomers of 2*H*-Azirin-3-amines.*

The preparation of gram-quantities of optically pure 2*H*-azirin-3-amines was achieved with the syntheses of the α-methylphenylalanine synthon **31**, and the isovaline synthon **32**, the diastereoisomeric mixtures of which could be separated by means of MPLC. In the case of the isovaline synthon, a phenylsulfonyl group had to be introduced to enable separation. Prior to the use of the synthon in peptide synthesis, the phenylsulfonyl group had to be removed (*Figure 9*) [70, 95, 96].



30 **31**

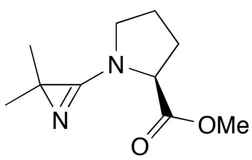
32 **33**

Figure 9. *Optically Pure 2*H*-Azirin-3-amines Prepared in Gram Quantities.*

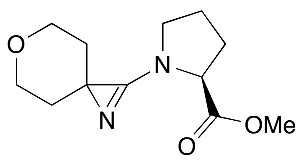
Recently, the series of optically pure synthons was significantly extended by the *N*-(1-naphtylethyl)-*N*-methyl-2*H*-azirin-3-amines **33** (*Figure 9*) [97, 98]. Although the naphtylethyl auxiliary group can be applied for a variety of optically pure synthons, the acidic hydrolysis of the terminal amide proceeds in some cases only in low yields.

3.3.4. Dipeptide Synthons

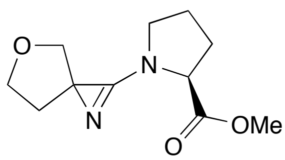
The Aib-Pro motif is widespread in peptaibols – in fact, 266 out of the 309 so far known peptaibol sequences contain the Aib-Pro motif [99]. However, the Aib-Pro amide bond is relative acid-labile, and therefore, it was of great interest to introduce this unit directly with a dipeptide synthon. Hence, the Aib-Pro dipeptide synthon **34** was synthesized (*Figure 10*) [69], and it has found application in the syntheses of model-peptides and the peptaibol *Trichovirin I 1B* [72]. In the meantime, further dipeptide synthons have become available such as, Thp-Pro (**35**) [66], Thf-Pro (**36**) [67], Aib-Hyp (**37**) [100], Aib-(Me)Ala (**38**) [101], Aib-(Me)Val (**39**) [101], Aib-HomoPro (**40**) [101], and Iva-Pro (**41**) [101] (*Figure 10*).



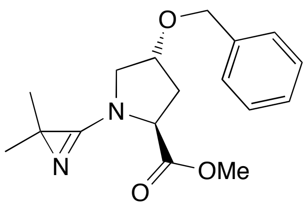
34 (Aib-Pro)



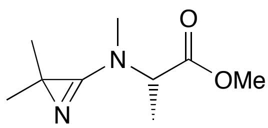
35 (Thp-Pro)



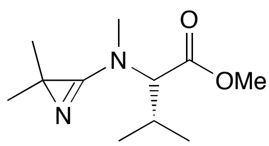
36 (Thf-Pro)



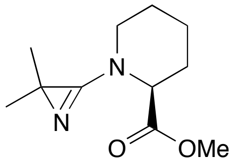
37 (Aib-Hyp)



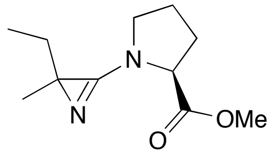
38 (Aib-(Me)Ala)



39 (Aib-(Me)Val)



40 (Aib-HomoPro)



41 (Iva-Pro)

Figure 10. *Dipeptide Synthons.*

3.4. Solid-Phase Peptide Synthesis

3.4.1. General

Construction of organic molecules, and in particular, peptides, on an insoluble solid support has obvious benefits. The separation of intermediates from soluble reagents and solvents can be achieved simply by filtration and washing the solid support. Therefore, also excess of reagents can be employed to drive the reactions to completion. Moreover, physical losses of material can be minimized as the molecule remains attached to the solid support throughout the synthesis. In particular in solid-phase peptide synthesis (SPPS), many reactions are simple, and therefore, can be automated. Solid-phase organic synthesis (SPOS) follows the principles of SPPS, although a broader range of reactions and linkers is applied.

However, the solid-phase approach also has its limitations. By-products arising from incomplete reactions, side reactions, or impure reagents will accumulate on the resin during the assembly of the molecule and contaminate the final product. The effect of incomplete reactions is dramatically illustrated in *Table 2*. Hence, product purification often is a challenge, and pure products can only be obtained by means of HPLC.

Table 2. *Yields of Final Product as a Function of Yield per Step and Number of Steps.*

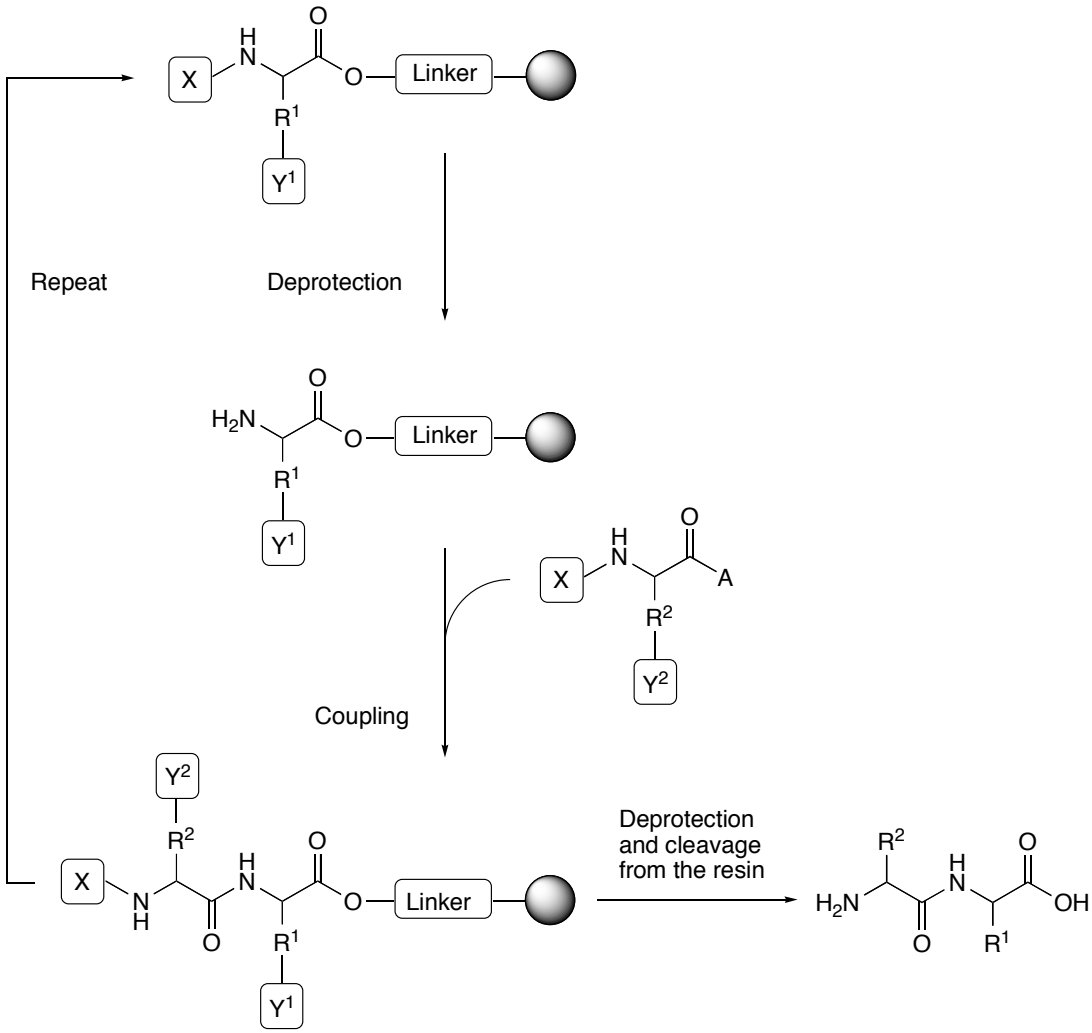
	Number of Steps			
Yield per Step	2	5	10	20
99%	98%	95%	90%	82%
95%	90%	77%	60%	36%
90%	81%	59%	35%	12%
85%	72%	44%	20%	< 1%
80%	64%	33%	11%	< 1%

Another drawback of solid-phase synthesis are the scanty possibilities of monitoring reactions or analyzing resin-bound intermediates. Although efforts were made in using NMR- and mass-spectroscopy, most often IR-spectroscopy and simple color-tests such as the *Kaiser*-(ninhydrin), the chloranil-, or the 2,4,6-trinitrosulfonic acid-test, are the sole tools for on-bead analysis. This may not imperatively affect routine and well documented syntheses, but can heavily complicate the development of new procedures.

In chapters 3.4.1 to 3.4.6 the *basic* principles of SPPS are discussed, and therefore, no specific literature is given. Instead, the reader is referred to some comprehensive books [102–106].

3.4.2. The Solid-Phase Principle

In standard SPPS the C-terminal amino acid of the target peptide is attached to an insoluble support *via* its carboxyl group (*Scheme 8*). The permanent protecting groups (Y) are used to mask the functional groups in the amino acid side chains. These permanent protecting groups may not be affected by any of the reaction conditions used to assemble the peptide chain. In contrast, the temporary protecting groups (X), masking the amino group of the amino acids to be introduced, have to be removed prior to the next coupling step.



Scheme 8. *The Principle of Solid-Phase Peptide Synthesis (SPPS).*

After having attached the first amino acid to the resin, the amino group of the first amino acid has to be deprotected. Excess of reagents and the released protecting group are removed by extensive washing of the resin. After coupling with the next amino acid (activated amino

acid or use of coupling reagents), excess of reagents can be removed again simply by washing the resin. Cleavage of the temporary protecting group introduces the next extension cycle. After having assembled the desired sequence, the peptide is released from the support and the side chain protecting groups are removed as well if desired.

3.4.3. Merrifield Solid-Phase Peptide Synthesis

Solid-phase peptide synthesis was introduced by *R. B. Merrifield* (Nobel Prize in Chemistry, 1984) in 1963: 'A novel approach to peptide synthesis has been the use of a chloromethylated polystyrene polymer as an insoluble but porous solid phase on which the coupling reactions are carried out. Attachment to the polymer constitutes protection of the carboxyl group (as a modified benzyl ester), and the peptide is lengthened from its amino-end by successive carbodiimide couplings. The method has been applied to the synthesis of a tetrapeptide, but incomplete reactions lead to the accumulation of by-products. Further development of this interesting method is awaited [107].' To many organic chemists this was both the beginning but also the end of an interesting new technique, since the difficulties in bringing heterogeneous reactions to completion would always result in impure products. Furthermore, the purification problems were expected to worsen as the chain length was increased beyond the described tetrapeptide.

But the situation changed dramatically when *R. B. Merrifield* published a second paper [108] reporting the successful synthesis of bradykinin. The nonapeptide was synthesized in four days and was isolated, purified and fully characterized in a further five. These results greatly exceeded any which could be achieved by contemporary solution-chemistry methods and set off high activity in SPPS.

Due to limited acid stability of the peptide-benzyl ester linkage, the original support (chloromethyl polystyrene) is rarely used now, and the C-terminal amino acid is attached to hydroxymethylphenylacetamidomethyl polystyrene (PAM resin) instead. In the *Merrifield*-approach the *tert*-butoxycarbonyl (Boc) group is used for temporary protection, while benzyl-based protecting groups are employed for side-chain protection. Removal of the Boc protecting group is usually performed with trifluoroacetic acid. Coupling is carried out with symmetrical anhydrides (PSA's) or benzotriazolyl esters of the incoming amino acids in *N*-methylpyrrolidone (NMP). Originally, the incoming amino acids were activated with *N*,*N'*-dicyclohexylcarbodiimide (DCC). Cleavage of the peptide from the resin and removal of the

side-chain protecting groups is effected with anhydrous hydrogen fluoride, which places special demands on the equipment.

3.4.4. Fmoc/^{t}Bu Solid-Phase Peptide Synthesis

Unlike the *Merrifield*-approach which achieves selectivity in the removal of temporary-protection, permanent-protection, and release of the peptide from the resin by graduated acidolysis, the Fmoc/^{t}Bu method is based on an orthogonal protecting group strategy. For temporary protection the base-labile [(9*H*-fluoren-9-yl)methyloxy]carbonyl (Fmoc) group is used, which can be removed by treatment with 20% piperidine in *N*,*N*-dimethylformamide (DMF). The removal, a β-elimination, proceeds usually fast ($\tau_{1/2}$ = 6 s), and can easily be monitored by UV-spectroscopy. In contrast, the side-chain protecting groups and the resin-linkage are acid-labile. For side-chain protection *tert*-butyl- and trityl-based protection groups were usually employed. Coupling is typically carried out in NMP with pre-formed active esters or with coupling reagents (chapter 3.2.1.).

3.4.5. Resins

In batchwise synthesis, the peptide containing resin is kept within a fritted reaction vessel. Reagents and solvents are added through the top of the vessel, and were removed by adding nitrogen pressure on the top, or applying vacuum to the bottom of the vessel. In general, the base matrix consists of divinylbenzene (typically 1%) cross-linked polystyrene, and can be functionalized by the *Friedel-Crafts* reaction with chloromethyl, aminomethyl, and benzhydrylamino groups. Because polystyrene is a hydrophobic, polarizable material, swelling is generally strong in dipolar aprotic solvents such as CH_2Cl_2, DMF, and NMP used for SPPS, but is poor in alkanes, protic solvents or water. Poly(ethyleneglycol)-polystyrene polymers (*e.g.*, Tentagel) on the other hand are more hydrophilic than pure polystyrene, and therefore, swell in a broad variety of solvents. The loading of commercially available Tentagel range between 0.15–0.30 mmol/g, which is considerably lower to that of pure polystyrene resins (*ca.* 0.4–1.5 mmol/g). Along with the low loading, the major disadvantage of Tentagel resins is the release of polyethyleneglycol (PEG) upon treatment with TFA or upon heating.

3.4.6. Linkers

In all variations of SPPS, the linker is of crucial importance, since it has to provide a reversible linkage between the synthetic molecule and the solid support. The linker should be stable to all reaction conditions used for the synthesis of the molecule, but finally should be cleavable not affecting the target molecule. In *Figure 11*, the linker resins most frequently used in *Merrifield*- and Fmoc/^{t}Bu-SPPS are listed. The chloromethyl and PAM resins are used for the *Merrifield*-approach, and cleavage is achieved with anhydrous hydrogen fluoride. The *Wang* resin is used in Fmoc/^{t}Bu SPPS, and peptide release is effected with trifluoroacetic acid (TFA) (90–95%) leading to unprotected peptide acids (apart from the N-terminus). Hyperacid labile linkers, *e.g.*, the *Rink* acid resin, the SASRIN resin, and the 2-chlorotrityl chloride resin, are able to be cleaved with a mild acidic solution (*ca.* 1% TFA in CH_2Cl_2) yielding protected peptide acids. For the preparation of peptide amides, the *Rink* amide resin and the hyperacid labile *Sieber* amide resin were developed. Photolabile linkers are discussed in more detail in the next chapter.

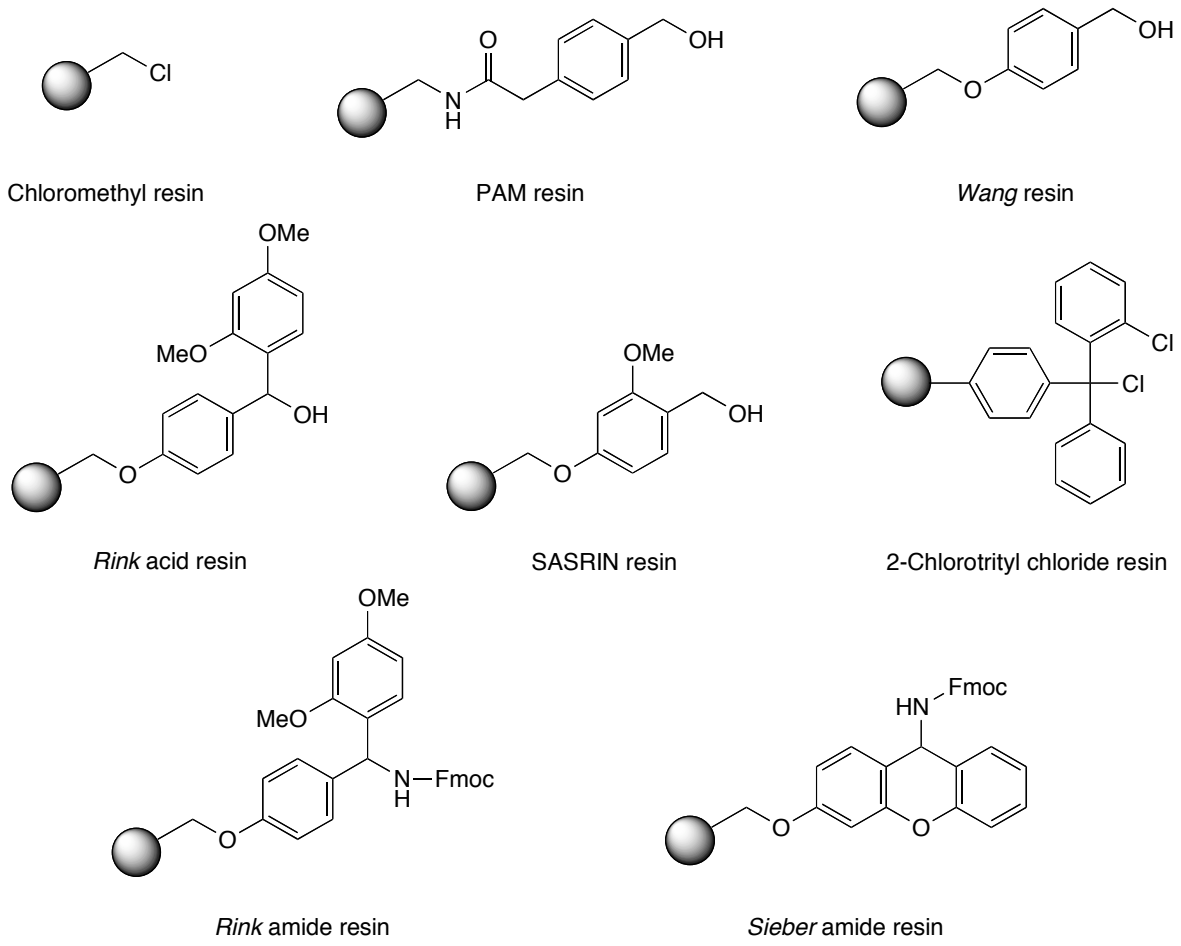
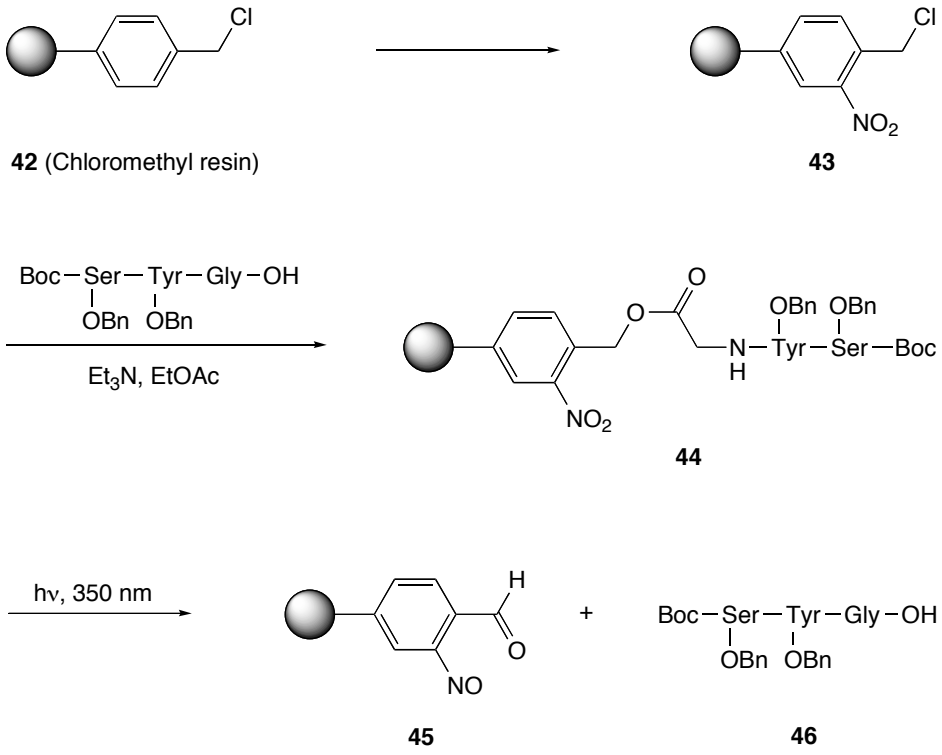


Figure 11. *Linker Resins Commonly Used for the Synthesis of Peptide Acids and Peptide Amides.*

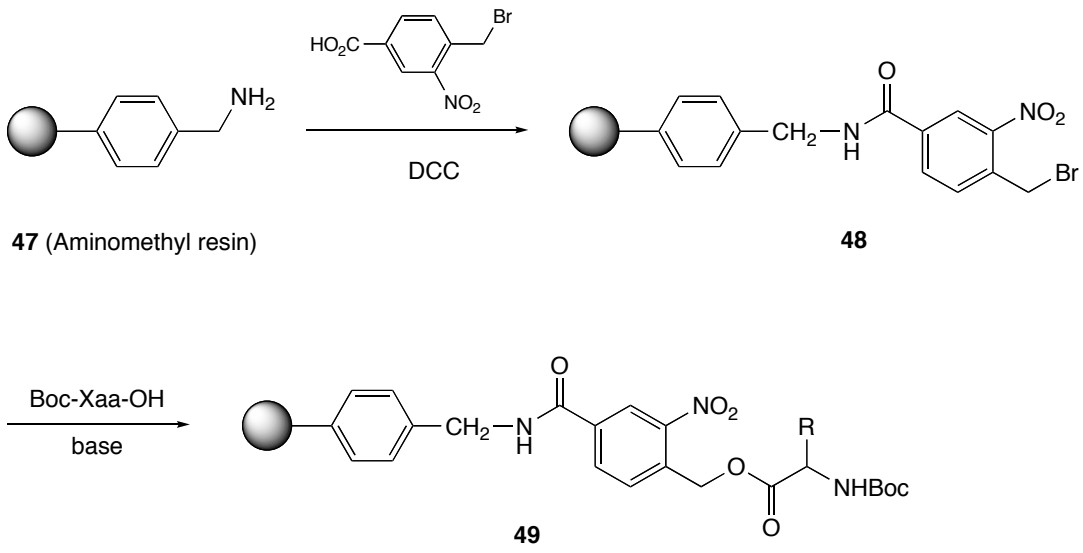
3.4.7. Photolabile Linkers

Photolabile linkers and protecting groups are orthogonal to acid-, base-, and transition metal-labile systems, and offer mild conditions for their cleavage [109–111]. The first photolabile linker to bind peptides to a solid support was introduced in 1973 [112], and was based on *o*-nitrobenzyl alcohol derivatives [113]. This photolabile resin was prepared by nitration of the already available chloromethyl resin (**42**) (*Scheme 9*). Heating the resulting nitrated resin **43** with an amino acid (or a peptide fragment) and a base led to the immobilization of the substrate. Photolysis at 350 nm gave the tripeptide in 62% yield, but the yield dropped significantly when releasing a tetrapeptide from the resin.



Scheme 9. *The First Photolabile Resin; Prepared by Nitration of Chloromethyl Resin.*

This problem was rationalized to poor swelling properties of the resin, which, in turn, were attributed to an increase in polarity by the numerous nitro groups. This issue was overcome by not nitrating the resin itself, but by introducing a proper linker, thus ensuring that only the required number of nitro groups were present. Hence, 4-bromomethyl-2-nitrobenzoic acid was attached to aminomethyl polystyrene resin (**47**), and the first amino acid was reacted with the benzylic bromide of resin **48** (*Scheme 10*) [114].



Scheme 10. *Introduction of a 'Proper' Linker*.

Although resin **48** provided a suitable support for longer peptides, it suffered from slow cleavage kinetics (12–24 h), and therefore undesired photo-oxidations occurred (*e.g.*, methionine to methionine sulfoxide). Incorporation of two alkoxy groups in the benzene ring to generate the veratryl-based linker **50a** increased the cleavage-rate dramatically (*Figure 12*) [115, 116]. An additional benzylic methyl group (**50b**) and the introduction of two additional methylene groups (**51**) further increased the rate. These nitroveratryl-based linkers are among the most effective photolinkers ever described, and they are stable to various chemical conditions. The half lives of these nitroveratryl-based linkers are in the range of a few minutes, while that of **52** (linker of original support **49**) was 362 minutes (in MeOH, 10 mW/cm^2 at 365 nm) [116].

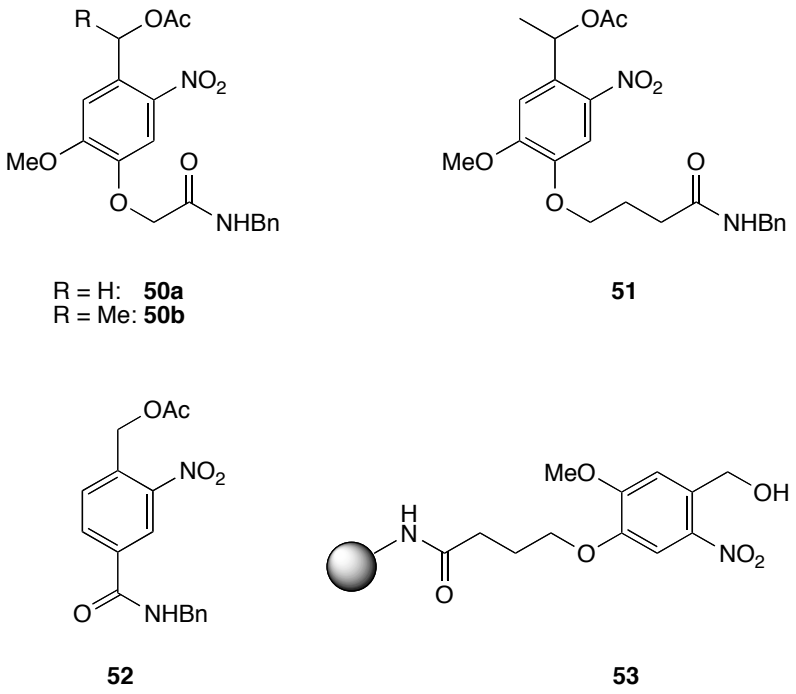


Figure 12. *Photolabile Linkers*.

The corresponding photocleavable resins (*e.g.*, **53**) are meanwhile commercially available. Cleavage is effected with UV-light at 365 nm in H_2O, DMSO, MeOH, or in a phosphate buffer (PBS)/dithiothreitol system for biological assays. For reasons of light scattering, shielding and shadowing effects of the resin, the photolysis of the supported linker is much slower than in the solution-phase. The addition of a scavenger (*e.g.*, ethanolamine, hydrazine, semicarbazide hydrochloride) during photolysis was found to be of crucial importance, otherwise the remaining nitrosoaldehyde resin may re-capture the amino group of the released molecule/peptide forming an imine [113, 117–119].

Nitroveratryl-based linkers have found successful application in the synthesis of peptides [115], small molecules such as thiazolidinones [120] and β-lactams [121], and oligonucleotides [122].

References

[1] D. Voet, J. G. Voet, in 'Biochemistry', John Wiley & Sons, New York, 2004.

[2] G. N. Ramachandran, C. Ramakrishnan, V. Sasissekharan, *J. Mol. Biol.* **1963**, *7*, 95.

[3] C. Ghélis, J. Yon, in 'Molecular Biology', Academic Press, New York, 1982.

[4] G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Prot. Chem.* **1985**, *37*, 1.

[5] C. Toniolo, *CRC Crit. Rev. Biochem.* **1980**, 1.

[6] W. F. DeGrado, *Adv. Prot. Chem.* **1988**, *39*, 51.

[7] V. J. Hruby, *Life Sci.* **1982**, *31*, 189.

[8] V. J. Hruby, F. Al-Obeidi, W. Kazmierski, *Biochem. J.* **1990**, *268*, 249.

[9] J. Rizo, L. M. Gierasch, *Annu. Rev. Biochem.* **1992**, *61*, 387.

[10] C.-F. Chang, M. H. Zehfus, *Biopolymers* **1996**, *40*, 609.

[11] B. Vitoux, A. Aubry, M. T. Cung, M. Marraud, *Int. J. Pept. Protein Res.* **1986**, *27*, 617.

[12] K. Gademann, M. Ernst, D. Seebach, *Helv. Chim. Acta* **2000**, *83*, 16.

[13] S. Abele, D. Seebach, *Eur. J. Org. Chem.* **2000**, 1.

[14] U. Koert, *Angew. Chem., Int. Ed.* **1997**, *36*, 1836.

[15] J. Rizo, M. Dhingra, L. M. Gierasch, in 'Molecular Conformation and Biological Interactions: G. N. Ramachandran Festschrift', Ed. P. Balaram and S. Ramaseshan, Indian Academy of Sciences, Bangalore, 1991, p. 469.

[16] L. J. Mathias, W. D. Fuller, D. Nissen, M. Goodman, *Macromolecules* **1978**, *11*, 534.

[17] W. C. Jones Jr., J. J. Nestor Jr., V. du Vigneaud, *J. Am. Chem. Soc.* **1973**, *95*, 5677.

[18] M. Goodman, M. Chorev, *Acc. Chem. Res.* **1979**, *12*, 1.

[19] R. W. Roeske, F. L. Weitl, K. U. Prasad, R. M. Thompson, *J. Org. Chem.* **1976**, *41*, 1260.

[20] A. F. Spatola, N. S. Agarwal, A. L. Bettag, J. A. Yankeelov Jr., C. Y. Bowers, W. W. Vale, *Biochem. Biophys. Res. Commun.* **1980**, *97*, 1014.

[21] R. G. Almquist, W.-R. Chao, M. E. Ellis, H. L. Johnson, *J. Med. Chem.* **1980**, *23*, 1392.

[22] M. T. Cox, J. J. Gormley, C. F. Hayward, N. N. Petter, *J. Chem. Soc., Chem. Commun.* **1980**, 800.

[23] D. Hudson, R. Sharpe, M. Szelke, *Int. J. Pept. Protein Res.* **1980**, *15*, 122.

[24] Y. Paterson, S. M. Rumsey, E. Benedetti, G. Némethy, H. A. Scheraga, *J. Am. Chem. Soc.* **1981**, *103*, 2947.

[25] H. Brückner, T. Kripp, M. Kiess, in 'Peptides 1990', Eds. E. Giralt, D. Andreu, ESCOM, Leiden, 1991, p. 387.

[26] C. Auvin-Guette, S. Rebuffat, Y. Prigent, B. Bodo, *J. Am. Chem. Soc.* **1992**, *114*, 2170.

[27] M. K. Das, S. Raghothamma, P. Balaram, *Biochemistry* **1986**, *25*, 7110.

[28] L. Whitmore, B. A. Wallace, *Nucleic Acid Research* **2004**, *32*, D593–D594.

[29] J. C. Sheehan, G. P. Hess, *J. Am. Chem. Soc.* **1955**, *77*, 1067.

[30] J. C. Sheehan, S. L. Ledis, *J. Am. Chem. Soc.* **1973**, *95*, 875.

[31] D. Sarantakis, J. Teichman, E. L. Lien, R. L. Fenichel, *Biochem. Biophys. Res. Commun.* **1976**, *73*, 336.

[32] D. Hudson, in 'Peptide Chemistry', Ed. Y. Kiso, Protein Research Foundation, Osaka, 1986, p. 4113.

[33] J. Izdebski, J. Pelka, in 'Peptides', Ed. U. Ragnarsson, 1985, p. 113.

[34] J. Izdebski, J. Bondaruk, S. W. Gumulka, P. Krzascik, *Int. J. Pept. Protein Res.* **1989**, *33*, 77.

[35] B. Castro, J. R. Dormoy, G. Evin, C. Selve, *Tetrahedron Letters* **1975**, *16*, 1219.

[36] J. Martinez, J. P. Bali, M. Rodriguez, B. Castro, R. Magous, J. Laur, M. F. Lignon, *J. Med. Chem.* **1985**, *28*, 1874.

[37] R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillessen, *Tetrahedron Letters* **1989**, *30*, 1927.

[38] L. A. Carpino, A. El-Faham, C. A. Minor, F. Albericio, *J. Chem. Soc., Chem. Commun.* **1994**, 201.

[39] L. A. Carpino, A. El-Faham, *J. Org. Chem.* **1994**, *59*, 695.

[40] O. Marder, *Chimica Oggi* **2002**, 37.

[41] J. Coste, E. Frerot, P. Jouin, B. Castro, *Tetrahedron Letters* **1991**, *32*, 1967.

[42] For a review about coupling methods for noncoded amino acids see: J. M. Humphrey, A. R. Chamberlin, *Chem. Rev.* **1997**, *97*, 2243.

[43] E. Frerot, J. Coste, A. Pantaloni, M.-N. Dufour, P. Jouin, *Tetrahedron* **1991**, *47*, 259.

[44] L. Kisfaludy, I. Schön, *Synthesis* **1983**, 325.

[45] E. Atherton, J. L. Holder, M. Meldal, R. C. Sheppard, R. M. Valerio, *J. Chem. Soc., Perkin Trans. 1* **1988**, 2887.

[46] A. Dryland, R. C. Sheppard, *Tetrahedron* **1988**, *44*, 859.

[47] W. D. Fuller, M. P. Cohen, M. Shabankareh, R. K. Blair, *J. Am. Chem. Soc.* **1990**, *112*, 7414.

[48] F. M. F. Chen, K. Kuroda, N. L. Benoiton, *Synthesis* **1978**, 928.

[49] E. P. Heimer, C.-D. Chang, T. Lambros, J. Meienhofer, *Int. J. Peptide Protein Res.* **1981**, *18*, 237.

[50] E. Fischer, E. Otto, *Ber. Dtsch. Chem. Ges.* **1903**, *36*, 2106.

[51] L. A. Carpino, B. J. Cohen, K. E. Stephens Jr., D. Sadat-Aalaee, J.-H. Tien, D. C. Langridge, *J. Org. Chem.* **1986**, *51*, 3732.

[52] M. Beyermann, M. Bienert, H. Niedrich, L. A. Carpino, D. Sadat-Aalaee, *J. Org. Chem.* **1990**, *55*, 721.

[53] L. A. Carpino, D. Sadat-Aalaee, M. Beyermann, *J. Org. Chem.* **1990**, *55*, 1673.

[54] M. Beyermann, D. Granitza, M. Bienert, M. Haussner, L. A. Carpino, in 'Peptides 1988', Eds. G. Jung, E. Beyer, Walter de Gruyter, Berlin, 1989, p. 28.

[55] M. Beyermann, M. Bienert, H. Niedrich, H. G. Chao, L. A. Carpino, in 'Peptides', Eds. J. Rivier, G. R. Marshall, ESCOM, Leiden, 1990, p. 925.

[56] L. A. Carpino, D. Sadat-Aalaee, H. G. Chao, R. H. DeSelms, *J. Am. Chem. Soc.* **1990**, *112*, 9651.

[57] J.-N. Bertho, A. Loffet, C. Pinel, F. Reuther, G. Sennyey, *Tetrahedron Lett.* **1991**, *32*, 1303.

[58] H. Wenschuh. M. Beyermann, E. Krause, L. A. Carpino, M. Bienert, *Tetrahedron Lett.* **1993**, *34*, 3733.

[59] H. Wenschuh, M. Beyermann, E. Krause, M. Brudel, R. Winter, M. Schümann, L. A. Carpino, M. Bienert, *J. Org. Chem.* **1994**, *59*, 3275.

[60] H. Wenschuh, M. Beyermann, H. Haber, J. K. Seydel, E. Krause, M. Bienert, L. A. Carpino, A. El-Faham, F. Alberico, *J. Org. Chem.* **1995**, *60*, 405.

[61] H. Heimgartner, *Angew. Chem., Int. Ed.* **1991**, *30*, 238.

[62] P. Wipf, Ph.D. thesis, University of Zurich, 1987.

[63] P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1988**, *71*, 140.

[64] P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1990**, *73*, 13.

[65] C. Strässler, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **1997**, *80*, 1528.

[66] G. Suter, S. A. Stoykova, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2000**, *83*, 2961.

[67] S. Stamm, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2003**, *86*, 1371.

[68] W. Altherr, H. Heimgartner, in 'Peptides 1990', Eds. E. Giralt, D. Andreu, ESCOM, Leiden, 1991, p. 107.

[69] R. T. N. Luykx, C. B. Bucher, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **1996**, *79*, 527.

[70] C. B. Bucher, H. Heimgartner, *Helv. Chim. Acta* **1996**, *79*, 1903.

[71] N. Pradeille, H. Heimgartner, *J. Pept. Sci.* **2003**, *9*, 827.

[72] R. T. N. Luykx, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2003**, *86*, 4093.

[73] N. Pradeille, O. Zerbe, K. Möhle, A. Linden, H. Heimgartner, *Chem. Biodiversity* **2005**, *2*, 1127.

[74] J. Lehmann, A. Linden, H. Heimgartner, *Tetrahedron* **1998**, *54*, 8721.

[75] J. Lehmann, H. Heimgartner, *Helv. Chim. Acta* **1999**, *82*, 1899.

[76] R. Breitenmoser, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2002**, *85*, 990.

[77] T. Jeremic, A. Linden, H. Heimgartner, *Chem. Biodiversity* **2004**, *1*, 1730.

[78] T. Jeremic, A. Linden, K. Möhle, H. Heimgartner, *Tetrahedron* **2005**, *61*, 1871.

[79] J. M. Villalgordo, H. Heimgartner, *Helv. Chim. Acta* **1997**, *80*, 748.

[80] K. N. Koch, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2000**, *83*, 233.

[81] K. N. Koch, H. Heimgartner, *Helv. Chim. Acta* **2000**, *83*, 1881.

[82] B. Iliev, A. Linden, R. Kunz, H. Heimgartner, *Tetrahedron* **2006**, *62*, 1079.

[83] M. Rens, L. Ghosez, *Tetrahedron Lett.* **1970**, *43*, 3765.

[84] K. Dietliker, H. Heimgartner, *Helv. Chim. Acta* **1983**, *66*, 262.

[85] R. A. Cherkasov, G. A. Kutyrev, A. N. Pudovik, *Tetrahedron* **1985**, *41*, 2567.

[86] M. P. Cava, M. J. Levinson, *Tetrahedron* **1985**, *41*, 5061.

[87] J. M. Villalgordo, H. Heimgartner, *Tetrahedron* **1993**, *49*, 7215.

[88] J. M. Villalgordo, H. Heimgartner, *Helv. Chim. Acta* **1993**, *76*, 2830.

[89] I. P. Piskunova, A. F. Ermeev, I. A. Vosekalna, *Tetrahedron* **1993**, *49*, 4671.

[90] J. A. Hyatt, *J. Org. Chem.* **1981**, *46*, 3953.

[91] H. Neunhoeffer, H. Ohl, *Chem. Ber.* **1978**, *111*, 299.

[92] G. J. Voghel, T. L. Eggerichs, B. Clamot, H. G. Viehe, *Chimia* **1976**, *30*, 191.

[93] W. Altherr, diploma thesis, University of Zurich, 1988.

[94] J. M. Villalgordo, Ph.D. thesis, University of Zurich, 1992.

[95] C. B. Bucher, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **1995**, *78*, 935.

[96] C. B. Bucher, Ph.D. thesis, University of Zurich, 1996.

[97] K. Brun, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2001**, *84*, 1756.

[98] K. Brun, Ph.D. thesis, University of Zurich, 2002.

[99] L. Whitmore, B. A. Wallace, *Nucleic Acid Research* **2004**, *32*, D593–D594.

[100] R. A. Breitenmoser, T. R. Hirt, R. T. N. Luykx, H. Heimgartner, *Helv. Chim. Acta* **2001**, *84*, 972.

[101] R. A. Breitenmoser, H. Heimgartner, *Helv. Chim. Acta* **2002**, *85*, 885.

[102] F. C. Dörwald, 'Organic Synthesis on Solid Phase: Supports, Linkers, Reactions', Wiley-VCH, Weinheim, 2000.

[103] E. Atherton, 'Solid Phase Peptide Synthesis: A Practical Approach', Eds. E. Atherton, R. C. Sheppard, Oxford University Press, New York, 1989.

[104] W. C. Chan, P. D. White, 'Fmoc Solid Phase Peptide Synthesis: A Practical Approach', Eds. W. C. Chan, P. D. White, Oxford University Press, New York, 2000.

[105] G. Jung, 'Combinatorial Chemistry: Synthesis, Analysis, Screening', Wiley-VCH, Weinheim, 1999.

[106] K. C. Nicolaou, R. Hanko, W. Hartwig, D. L. Coffen, J. E. A. Luithle, R. Haag, A. Hebel, J.-F. Stumbé, S. Bräse, S. Dahmen, 'Handbook of Combinatorial Chemistry', Eds. K. C. Nicolaou, R. Hanko, W. Hartwig, Wiley-VCH, Weinheim, 2002.

[107] R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149.

[108] R. B. Merrifield, *J. Am. Chem. Soc.* **1964**, *86*, 304.

[109] For a review on the use of photolabile protecting groups and linkers see: C. G. Bochet, *J. Chem. Soc., Perkin Trans. 1* **2002**, 125.

[110] For a review on solid-phase peptide synthesis including photolabile supports see: P. Lloyd-Williams, F. Albericio, E. Giralt, *Tetrahedron* **1993**, *49*, 11065.

[111] For a review on the use of photolabile protecting groups see: V. N. R. Pillai, *Synthesis* **1980,** 1.

[112] D. H. Rich, S. K. Gurwara, *J. Chem. Soc., Chem. Commun.* **1973**, 610.

[113] A. Patchornik, B. Amit, R. B. Woodward, *J. Am. Chem. Soc.* **1970**, *92*, 6333.

[114] D. H. Rich, S. K. Gurwara, *J. Am. Chem. Soc.* **1975**, *97*, 1575.

[115] C. P. Holmes, D. G. Jones, *J. Org. Chem* **1995**, *60*, 2318.

[116] C. P. Holmes, *J. Org. Chem.* **1997**, *62*, 2370.

[117] D. B. Henriksen, K. Breddam, O. Buchardt, *Int. J. Pept. Protein Res.* **1993**, *41*, 169.

[118] D. Ramesh, R. Wieboldt, A. P. Billington, B. K. Carpenter, G. P. Hess, *J. Org. Chem.* **1993**, *58*, 4599.

[119] V. N. R. Pillai, M. Mutter, E. Bayer, *Tetrahedron Lett.* **1979**, *20*, 3409.

[120] C. P. Holmes, J. P. Chinn, G. C. Look, E. M. Gordon, M. A. Gallop, *J. Org. Chem.* **1995**, *60*, 7328.

[121] B. Ruhland, A. Bhandari, E. M. Gordon, M. A. Gallop, *J. Am. Chem. Soc.* **1996**, *118*, 253.

[122] H. Venkatesan, M. M. Greenberg, *J. Org. Chem.* **1996**, *61*, 525.

4. The 'Azirine/Oxazolone Method' under Solid-Phase Conditions[1]

Aib-containing peptides are synthesized by means of the 'azirine/oxazolone method' on solid-phase from the N- to the C-terminus. In this new and convenient method for the synthesis of sterically demanding peptides on solid-phase, 2*H*-azirin-3-amines are used to introduce α-aminoisobutyric acid, an α,α-disubstituted α-amino acid into the peptide without the need for further reagents. Using this method, segments of naturally occuring peptaibols have been prepared.

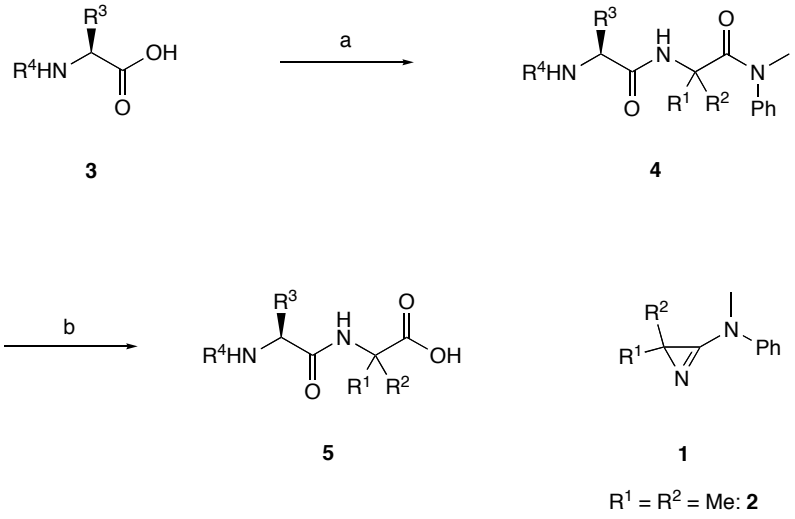
1. Introduction

Solid-phase peptide synthesis (SPPS) allows rapid access to peptides and peptide conjugates.[1-3] In particular, its importance appears in combinatorial chemistry, where it made the synthesis of compound libraries possible. SPPS is exclusively carried out from the C- to the N-terminus. Attempts to reverse the strategy failed mainly due to incomplete couplings and significant epimerization during the coupling steps. Therefore, the synthesis of C-terminal modified peptides and peptide libraries is not routine. Nevertheless, methods for the synthesis of peptides from the N- to the C-terminus were investigated.[4-9] Moreover, the introduction of sterically demanding α,α-disubstituted α-amino acids into peptides on solid-phase still remains a challenge, although progress has been made in the case of aminoisobutyric acid (Aib) and isovaline (Iva).[10-12]

Peptides containing α,α-disubstituted α-amino acids are restricted in their conformational freedom.[13-16] As a consequence of the rigidity of the peptide backbone, secondary structures such as β-turns and helices are stabilized or even promoted.[17-20] The synthesis of such conformationally restricted peptides is a basic approach when searching for the biologically active conformation of a peptide. A useful method for introducing α,α-disubstituted α-amino acids into peptides is the 'azirine/oxazolone method', in which 2*H*-azirin-3-amines **1** are used as amino acid synthons (Scheme 1).[21-23] Thus, the reaction of 2*H*-azirin-3-amines, e.g., the Aib synthon **2**, with amino or peptide acids **3** leads to peptide amides **4**, the terminal amide

[1] S. Stamm, H. Heimgartner, *Eur. J. Org. Chem.* **2004**, 3820.

bond of which can be hydrolyzed selectively to give extended peptide acids **5**. In solution-phase chemistry, the 'azirine/oxazolone method' was successful at introducing a multiplicity of sterically demanding α,α-disubstituted α-amino acids into peptides and found successful application in the synthesis of some antibiotic active peptaibols or segments of it.[24-28] A drawback of this method was that it was not yet applicable to solid-phase synthesis.



3 **4** **5** **1**

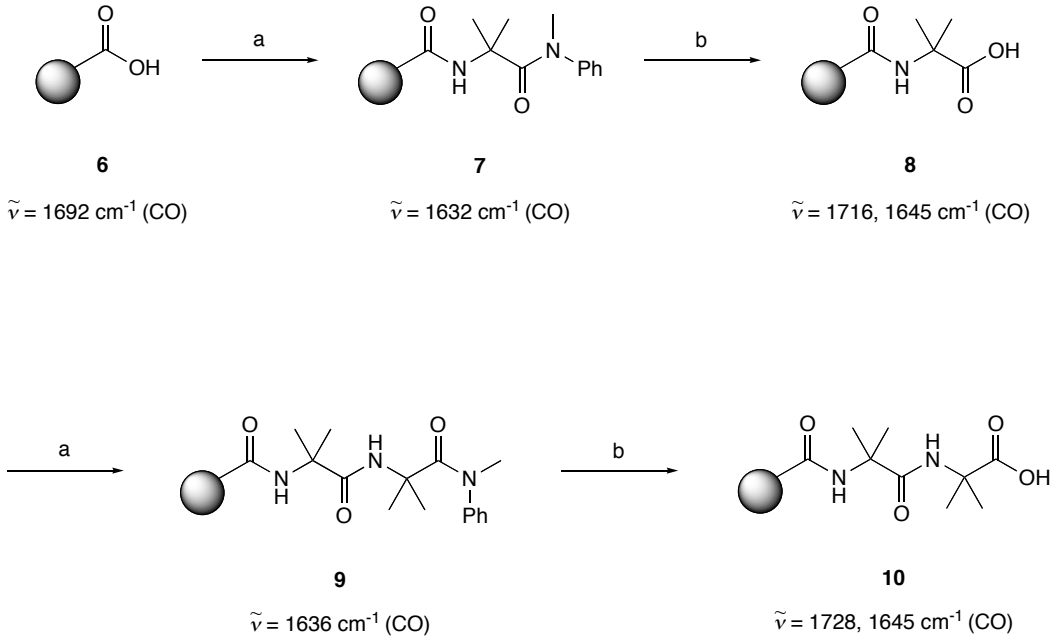
$R^1 = R^2$ = Me: **2**

Scheme 1. a) **1**, CH_2Cl_2; b) HCl (3M), THF/H_2O.

Here we report the successful attempt to synthesize peptides by means of the 'azirine/oxazolone method' on solid-phase – a synthesis from the N- to the C-terminus, where 2*H*-azirin-3-amines are used to introduce α,α-disubstituted α-amino acids into peptides without the need for further reagents.

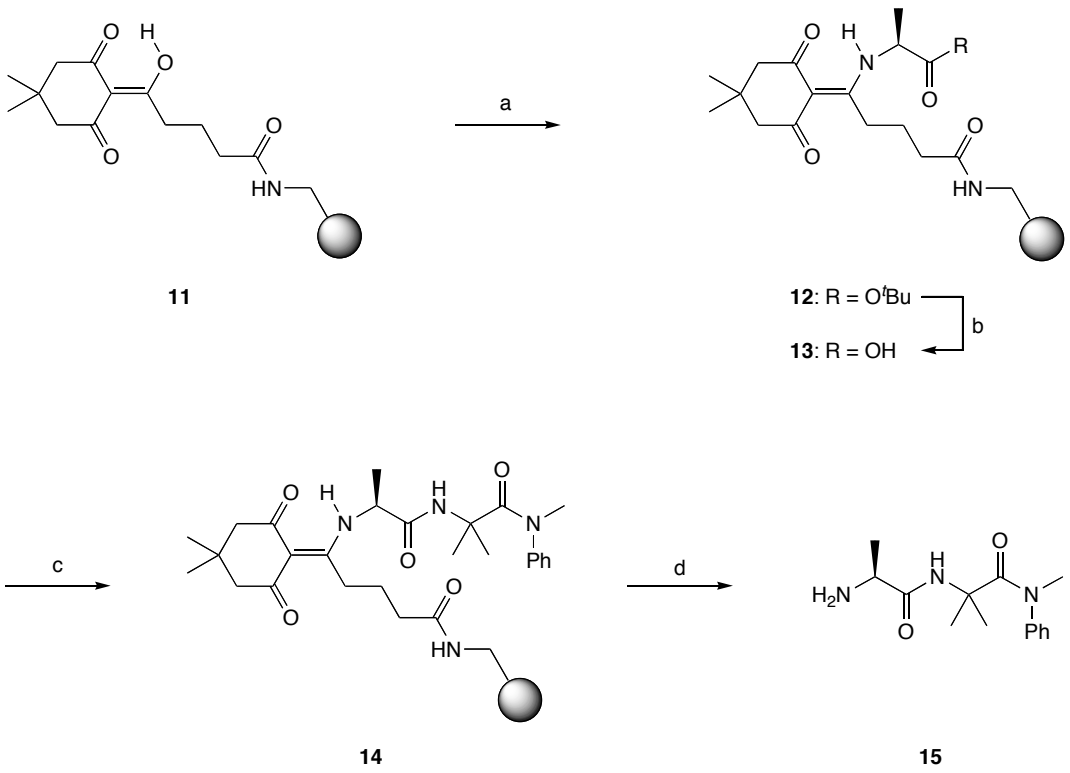
2. Results and Discussion

The first questions we asked concerned chemical reactivity: Do 2*H*-azirin-3-amines react in a similar way on a solid support as in solution, i.e. is a terminal amide formed by the reaction of a 2*H*-azirin-3-amine with a solid-phase bound carboxylic acid? Is the selective hydrolysis of the terminal amide possible? Attenuated total reflectance (ATR) FT-IR measurements of the corresponding intermediates **7**, **8**, **9** and **10** (Scheme 2), as well as the synthesis of a resin-bound tripeptide and its hydrolysis[29] confirmed our assumptions.



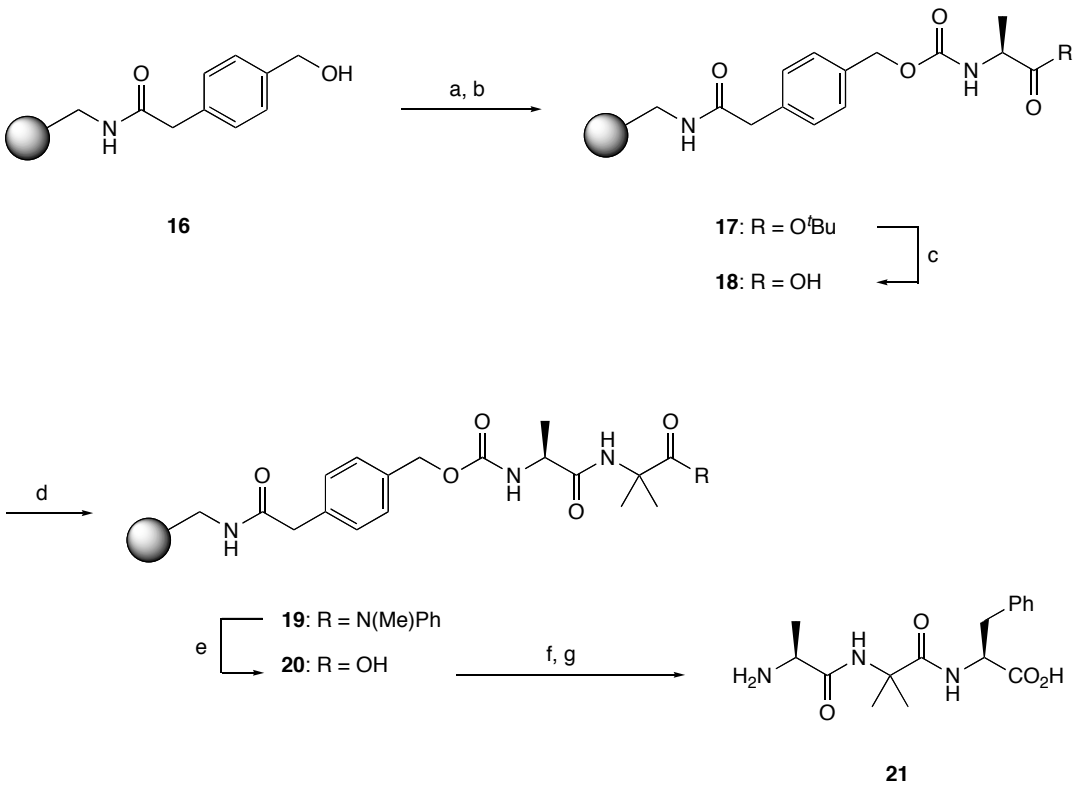
Scheme 2. a) **2**, CH_2Cl_2; b) HCl (3M), THF/H_2O.

In all variations of solid-phase synthesis, the linker is of crucial importance. Therefore, this was the second problem we dealt with. The 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde)-linker, developed by Bycroft et al.,[30] is stable in trifluoroacetic acid (TFA), but can be cleaved with 2% N_2H_4 in DMF. Its use led to the successful synthesis of H-Ala-Aib-N(Me)Ph (**15**, Scheme 3). However, the selective hydrolysis of the terminal amide group of **14** was not possible, since the Dde-linker was not stable in aqueous, acidic medium.



Scheme 3. a) H-Ala-O^{t}Bu · HCl, CH_2Cl_2, DIPEA; b) TFA/CH_2Cl_2 (1:1), TIPS; c) **2**, CH_2Cl_2; d) N_2H_4 (2%), DMF. DIPEA = *N*,*N*-diisopropylethylamine, TFA = trifluoroacetic acid, TIPS = triisopropylsilane, DMF = *N*,*N*-dimethylformamide.

A wide variety of amines were immoblizied on solid supports using carbamate linkers, which were synthesized either with phosgene,[31-34] 1,1'-carbonyldiimidazole (CDI)[35] or 4-nitrophenylchloroformate.[36-39] The use of a carbamate linker to attach amino acids via their N-terminus was first reported by Letsinger.[5, 31] Therefore, we also planned to use a carbamate linker, but the stability of the carbamate had to be tuned carefully. The carbamate has to be stable during hydrolysis of the terminal amide with 3M HCl and deprotection of the ^{t}Bu ester with TFA, but finally should be cleavable under conditions not affecting the peptide. It was shown that the carbamate linker formed from [4-(hydroxymethyl)phenyl]acetamidomethyl (PAM) resin **16** fulfilled these requirements the best (Scheme 4).[40]



Scheme 4. a) $COCl_2$ (1.9M in PhMe), THF; b) H-Ala-O^{t}Bu · HCl, DIPEA, CH_2Cl_2; c) TFA, CH_2Cl_2, TIPS; d) **2**, CH_2Cl_2; e) HCl (3M), THF/H_2O; f) H-Phe-O^{t}Bu · HCl, PyBOP, DIPEA, CH_2Cl_2; g) HBr (33%) in acetic acid. PyBOP = (1*H*-benzotriazol-1-yloxy)tripyrrolidino-phosphonium hexafluorophosphate.

Phosgene in toluene (1.9M) was added to PAM resin **16** to generate a chloroformate intermediate. Reaction with H-Ala-O^{t}Bu afforded resin **17**. Amino acid ^{t}Bu esters proved to be useful building blocks for inverse peptide synthesis,[4] because their chemistry is well known and they are readily available from commercial suppliers. Deprotection with TFA afforded resin **18**, which was treated with a solution of *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**2**, 4 equiv.) in DCM (conc. of **2** = 0.2M). Unconsumed **2** can easily be recovered and used in further coupling steps. Hydrolysis of resin **19** with 3M HCl in H_2O/THF afforded resin **20**, which was conventionally coupled to H-Phe-O^{t}Bu using PyBOP as coupling reagent. Cleavage from the solid support was achieved with HBr (33%) in acetic acid. After purification by means of HPLC and lyophilization, model peptide **21** was isolated in 50% yield. A second model peptide (H-Ala-Aib-Val-Aib-Phe-OH (**22**)) containing two Aib

residues was synthesized in an analogues manner (Table 1) with 33% yield after HPLC and lyophilization.

Table 1. General procedures.

Description	Reagents[a]	Duration and repetition
General procedure for the attachment of the first amino acid		
Chloroformate formation	10 eq. $COCl_2$ (1.9M) in toluene, THF	1 × 2 h
Washes	THF, CH_2Cl_2	2 ×, each
Coupling	4 eq. H-AA-O^{t}Bu · HCl, 8 eq. DIPEA, CH_2Cl_2	1 ×
Washes	CH_2Cl_2, DMF, CH_2Cl_2	3 ×, each
General procedure for removing the ^{t}Bu protecting group		
Hydrolysis	TFA/CH_2Cl_2, TIPS (5 %)	1 × 5 s (25 %), 1 × 30 min (50 %)
Washes	CH_2Cl_2, DMF, CH_2Cl_2	3 ×, each
General procedure for peptide synthesis using 2*H*-azirin-3-amine 2		
Coupling	4 eq. **2**, CH_2Cl_2	1 ×
Washes	CH_2Cl_2	3 ×
Hydrolysis	HCl (3M) in THF/H_2O	1 ×
Washes	THF, DMF, CH_2Cl_2	3 ×, each
General procedure for peptide synthesis using coupling reagents method A, (removing ^{t}Bu see above)		
Coupling	4 eq. PyBOP, 4 eq. H-AA-O^{t}Bu · HCl, 12 eq. DIPEA, CH_2Cl_2	1 ×
Washes	CH_2Cl_2, DMF, CH_2Cl_2	2 ×, each
General procedure for peptide synthesis using coupling reagents method B,[47] (removing ^{t}Bu see above)		
Coupling	6 eq. HOBt, 4 eq. PyBOP, 4 eq. H-AA-O^{t}Bu · HCl, 11 eq. NMM, DMF	1 ×
Washes	CH_2Cl_2, DMF, CH_2Cl_2	2 ×, each
General procedure for cleavage from the support		
Cleavage	HBr (33 %) in acetic acid, 2 drops of H_2O	1 × 6 h
Washes	Acetic acid/CH_2Cl_2 (1:1), CH_3CN/CH_2Cl_2 (1:1)	3 ×, each

[a] DIPEA = *N*,*N*-diisopropylethylamine, HOAt = 1-hydroxy-7-azabenzotriazole, HOBt = 1-hydroxybenzotriazole, NMM = *N*-methylmorpholine, PyBOP = (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, TIPS = triisopropylsilane.

A crucial point in the context of this strategy was the question of epimerization of the C(α) center(s). In order to determine the extent of racemization/epimerization, the tripeptide **21** was hydrolyzed and the amino acids were analyzed by means of capillary gas chromatography with enantiomer labeling.[41] As a result, alanine and phenylalanine have racemized by 0.6% and 2.8%, respectively. It was not possible to achieve resolution of the epimers by means of HPLC[42] on the crude product, the derivatized crude product[43] or the purified product. Therefore, we assume that the extent of racemization/epimerization in the crude product is similar to that of the purified product, i.e. the synthesis has been carried out with an acceptable degree of racemization, comparable to that seen in classical SPPS.

To examine the use of the 'azirine/oxazolone method' on solid-phase, we attempted to synthesize a *Peptaibolin* derivative and two segments of other peptaibols.[44] The hexapeptide H-Ala-Aib-Ala-Gln-Aib-Val-OH (A4–9) (**23**) of the peptaibol antibiotic *Alamethicin*[45, 46] was synthesized using the procedures listed in Table 1 in 25% yield (Table 2). Both Aib residues were introduced by means of the 'azirine/oxazolone method', Ala (A3) by method A (Table 1) and Gln as well as Val according to method B.[47] Method B was applied to prevent nitrile formation as the side chain of Gln was not protected.[48] The pentapeptide H-Leu-Aib-Leu-Aib-Phe-OH (**24**) is a derivative of *Peptaibolin*[49] (Ac-Leu-Aib-Leu-Aib-Pheol), and it was synthesized according to the procedures listed in Table 1. Both Aib residues were introduced by means of the ‚azirine/oxazolone method', while method A was used for the coupling of the other amino acids. The lower yield (20%) is the result of incomplete coupling of phenylalanine, but a repeated coupling with additional pentafluorophenol did not increase the yield. The segment H-Val-Aib-Gly-Aib-Ala-OH (A9–13) (**25**) of *Stilboflavin A3*[50] was prepared in 30% yield using the 'azirine/oxazolone method' and method A.

Table 2. Synthesized peptides.

Description	Sequence	Yield [%][a]
Modelltripeptide	H-Ala-Aib-Phe-OH (**21**)	50
Modellpentapeptide	H-Ala-Aib-Val-Aib-Phe-OH (**22**)	33
A4–9 of *Alamethicin*	H-Ala-Aib-Ala-Gln-Aib-Val-OH (**23**)	25
Peptaibolin derivative	H-Leu-Aib-Leu-Aib-Phe-OH (**24**)	20
A9–13 of *Stilboflavin A3*	H-Val-Aib-Gly-Aib-Ala-OH (**25**)	30

[a] Yield of product isolated after HPLC purification.

3. Conclusions

In conclusion, we were able to adapt the 'azirine/oxazolone method' on solid-phase, and consequently developed a new and convenient method for the synthesis of sterically demanding peptides on solid-phase. The simple procedure can possibly be automated. It has been shown that this approach for the synthesis of Aib-containing peptides from the N- to the C-terminus can be combined with conventional coupling methods. The method found a successful application in the synthesis of different peptaibol segments.

4. Experimental Part

4.1. General Remarks

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Hydroxymethylpolystyrene, 1% divinylbenzene, 100-200 mesh, loading 0.98 mmol/g from *Novabiochem* (*Calbiochem-Novabiochem*, Läufelfingen, Switzerland). [4-(Hydroxymethyl)phenyl]acetamidomethylpolystyrene (4-(oxomethyl)phenylacetamidomethylpolystyrene), 1% divinylbenzene, 100-200 mesh, loading 0.62 mmol/g from *Acros Organics* (*Acros Organics*, Geel, Belgium). Carboxylpolystyrene, 1% divinylbenzene, 200-400 mesh, loading 4.0 mmol/g from *Lipal Biochemicals* (*Lipal Biochemicals*, Gundetswil, Switzerland). Aminomethylpolystyrene, 1% divinylbenzene, 100-200 mesh, loading 1.14 mmol/g, hydroxyethylpolystyrene, 1% divinylpolystyrene, 100-200 mesh, loading 1.30 mmol/g, and carboxylpolystyrene, 1% divinylbenzene, 100-200 mesh, loading 1.96 mmol/g from *Rapp Polymere* (*Rapp Polymere*, Tübingen, Germany). *N*,2,2-Trimethyl-*N*-phenyl-2*H*-azirin-3-amine was synthesized according to the method of Villalgordo and Heimgartner.[51, 52] Reaction vessels for solid phase synthesis: Single fritted (20 μm) PE reservoir (15 mL) (*Separtis*, Grenzach-Wyhlen, Germany) were used on an *Advanced ChemTech PLS 4 × 6* Shaker (*Advanced ChemTech, Inc.*, Louisville, KY, USA) with a selfmade adapter. The original *Advanced ChemTech* reaction vessels were used for reactions under N_2. High-performance liquid chromatography (HPLC): instrument: *Waters 600E* multisolvent delivery system equipped with a *Waters 996 PDA* (*Waters*, Milford, CA, USA); column: *Interchim Uptisphere ODB C18*, 300 Å, 10 μm, 250 × 4.6 mm (*Interchim*, Montluçon, France), *Interchim Uptisphere WOD C18*, 300 Å, 10 μm, 250 × 21.2 mm (prep. HPLC) or *Vydac 218TP C18*, 300 Å, 10 μm, 250 × 22 mm (prep. HPLC) (*Vydac*, Hesperia, CA, USA). Column chromatography (CC): Silica gel *C-560* (0.04-0.063 mm, 230-400 mesh)

from *Chemie Uetikon* (*CU Chemie Uetikon GmbH*, Uetikon, Switzerland). Prep. TLC: *Merck* TLC plates (glass), silica gel *60* F_{254}, 0.25 mm (*Merck KGaA*, Darmstadt, Germany). IR Spectra: *Perkin-Elmer*, *Spectrum one FT-IR* spectrophotometer (*Perkin-Elmer*, Wellesley, MA, USA); ATR-FT-IR on a *Bio-Rad FTS-45* (*Bio-Rad*, Hercules, CA, USA) instrument equipped with a *MKII Golden Gate* single reflection ATR system from *Specac* (*Specac Inc.*, Smyrna, GA, USA). NMR Spectra: *Bruker ARX-300*, *Bruker DRX-500* or *Bruker DRX-600* (*Bruker Biospin*, Karlsruhe, Germany). Chemical shifts are given in ppm relative to tetramethylsilane (TMS) as internal standard. 2D-NMR experiments were performed for assignment of the signals. Some spin systems were simulated with *NMR-Sim* from *Bruker*. HPLC-MS: instrument: *Hewlett-Packard* HP 1100 HPLC system (*Hewlett-Packard*, Palo Alto, CA, USA) equipped with a *HTS PAL* autosampler (*CTC Analytics*, Zwingen, Switzerland), connected to a *Bruker ESQUIRE-LC* quadrupole ion trap instrument (*Bruker Daltonik GmbH,* Bremen, Germany) equipped with a combined *Hewlett-Packard* Atmospheric Pressure Ion (API) source (*Hewlett-Packard Co.*, Palo Alto, CA, USA); column: *Interchim Uptisphere HDO C18*, 120 Å, 3 μm, 200 × 2.0 mm column (*Interchim*, Montluçon, France); eluents: A = H_2O/HCOOH (99.95/0.05), B = CH_3CN/HCOOH (99.95/0.05); flow rate: 0.18 mL/min, gradient (A:B): 0-10 min: 95:5-50:50, 10-20 min: 50:50-0:100, 20-30: 0:100. MS: *Bruker ESQUIRE-LC* quadrupole instrument (*Bruker Daltonik GmbH,* Bremen, Germany) or *Finnigan TSQ-700* triple quadrupole instrument (*Finnigan* MAT, San Jose, CA, USA). Direct infusion ESI-MS were performed with a syringe infusion pump at a flow rate of 5 μL/min.

4.2. Abbreviations

Aib: α-aminoisobutyric acid; ATR-FT-IR: attenuated total reflectance fourier transform infrared spectroscopy; CC: column chromatography; DCM: dichloromethane; Dde: *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl; DIPCDI: diisopropylcarbodiimid; DIPEA: *N*,*N*-diisopropylethylamine; HM: hydroxymethyl; HOAt: 1-hydroxy-7-azabenzotriazole; HOBt: 1-hydroxybenzotriazole; NMM: *N*-methylmorpholine; PAM: [4-(hydroxy-methyl)phenyl]acetamidomethyl; PfpOH: pentafluorophenol; PyBOP: (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TIPS: triisopropylsilane.

4.3. Synthesis of Resin 10, IR Analysis

Resin **7**. Carboxylpolystyrene (50 mg, 0.20 mmol) was swollen in DCM. A solution of *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**2**, 178 mg, 1.02 mmol) in DCM (1.5 mL) was added and the resin agitated at r.t. for 15 h. The resin was separated by filtration, washed with DCM (3×) and Et_2O (3×) and dried in vacuum. ATR-FT-IR (beads): $\tilde{\nu}$ = 1632 cm^{-1} (carboxylpolystyrene, beads: $\tilde{\nu}$ = 1692 cm^{-1}). *Resin* **8**. Resin **7** was swollen in THF. A solution of HCl (2 mL, 3 M in THF/H_2O, prepared from conc. HCl and THF) was added and the resin agitated at r.t. for 16 h. The resin was separated by filtration, washed with THF (3×), DCM (3×) and Et_2O (3×) and dried in vacuum. ATR-FT-IR (beads): $\tilde{\nu}$ = 1716, 1645 cm^{-1}. *Resin* **9**. Remaining resin **8** (ca. 5 mg) was swollen in DCM. A solution of **2** (13 mg, 0.08 mmol) in DCM (1.5 mL) was added and the resin agitated at r.t. for 16 h. The resin was separated by filtration, washed with DCM (3×) and Et_2O (3×) and dried in vacuum. ATR-FT-IR (beads): $\tilde{\nu}$ = 1636 cm^{-1}. *Resin* **10**. Resin **9** was swollen in THF. A solution of HCl (2 mL, 3 M in THF/H_2O, prepared from conc. HCl and THF) was added and the resin agitated at r.t. for 7 h. The resin was separated by filtration, washed with THF (3×), DCM (3×) and Et_2O (3×) and dried in vacuum. ATR-FT-IR (beads): $\tilde{\nu}$ = 1728, 1645 cm^{-1}.

4.4. Synthesis of Resin-bound Ala-Aib-Phe-O^{t}Bu and its Hydrolysis

Carboxylpolystyrene (103 mg, 0.20 mmol) was swollen in DCM. A solution of H-Ala-O^{t}Bu · HCl (76 mg, 0.42 mmol) in DCM (1 mL), a solution of PyBOP (208 mg, 0.40 mmol) in DCM (1 mL), and DIPEA (0.2 mL, 1.17 mmol) were added and the resin agitated at r.t. over night. The resin was separated by filtration, washed with DMF (4×), DCM (3×) and Et_2O (2×), and dried in vacuum. ATR-FT-IR (beads): $\tilde{\nu}$ = 1729, 1663, 1652 cm^{-1} (carboxylpolystyrene, beads: $\tilde{\nu}$ = 1685 cm^{-1}). The new resin was swollen in DCM. TFA/DCM (3 mL, 1:1) and TIPS (0.2 mL) were added and the resin agitated for 2 h. The resin was separated by filtration, washed with DMF (3×), DCM (3×) and Et_2O (2×), and dried in vacuum. ATR-FT-IR (beads): $\tilde{\nu}$ = 1733, 1641 cm^{-1}. Half of the obtained resin was swollen in DCM. A solution of *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**2**, 40 mg, 0.23 mmol) in DCM (2 mL) was added and the resin agitated at r.t. over night. The resin was separated by filtration, washed with DCM (3×) and Et_2O (2×), and dried in vacuum. ATR-FT-IR (beads): $\tilde{\nu}$ = 1637 cm^{-1}. The resulted resin was swollen in THF. A solution of HCl (3 mL, 3M in THF/H_2O, prepared from conc. HCl and THF) was added and the resin agitated at r.t. over night. The resin was separated by filtration, washed with THF (3×), DCM (3×) and Et_2O (2×), and dried in vacuum. ATR-FT-IR (beads): $\tilde{\nu}$ = 1719, 1636 cm^{-1}. The resin was swollen in

DCM. A solution of PyBOP (103 mg, 0.20 mmol) in DCM (1 mL), H-Phe-O^{t}Bu · HCl (51 mg, 0.20 mmol) in DCM (1 mL) and DIPEA (0.1 mL, 0.58 mmol) were added and the resin agitated at r.t. over night. The resin was separated by filtration, washed with DMF (3×), DCM (3×) and Et_2O (2×), and dried in vacuum. ATR-FT-IR (beads): $\tilde{\nu}$ = 1730, 1652, 1646 cm^{-1}. The resin-bound peptide was hydrolyzed according to Westall et al.[53] (propionic acid/HCl (1:1) at 130°C, sealed tube). The mixture was filtered, the filtrate was concentrated and dried in vacuum to yield a colorless powder. MS (ESI): *m*/*z* (%): 90 (2) $[Ala+H]^+$, 104 (17) $[Aib+H]^+$, 120 (60),[54] 166 (100) $[Phe+H]^+$. ATR-FT-IR (beads): $\tilde{\nu}$ = 1686 cm^{-1}.

4.5. Synthesis of H-Ala-Aib-N(Me)Ph (15) Using the Dde-Linker

The immobilised linker system **11** was synthesized according to Chhabra et al.[55] using aminomethypolystyrene (500 mg, 0.57 mmol), 5-(4,4-dimethyl-2,6-dioxocyclohexylidene)-5-hydroxypentanoic acid (291 mg, 1.14 mmol), DIPCDI (177 μL, 1.14 mmol) and HOAt (2.28 mL, 1.14 mmol of a 0.5 M solution in DMF). *Resin* **12**. Resin **11** was swollen in DMF, then H-Ala-O^{t}Bu · HCl (416 mg, 2.29 mmol) and DIPEA (390 μL, 2.28 mmol) in DMF (5 mL) were added and the resin was agitated at r.t. for 1 d. The resin was separated by filtration and washed with DMF (3×) and DCM (3×). *Resin* **13**. Resin **12** was swollen in DCM. TFA/DCM (5 mL, 1:1) and TIPS (230 μL) were added and the resin agitated for 2 h. The resin was separated by filtration and washed with DCM (3×), DMF (3×) and DCM (2×). *Resin* **14**. Resin **13** was swollen in DCM. A solution of *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**2**, 204 mg, 0.17 mmol) in DCM (4 mL) was added and the resin agitated at r.t. over night. The resin was separated by filtration and washed with DCM (3×). *H-Ala-Aib-N(Me)Ph (***15***)*. Resin **14** was swollen in DMF. A solution of $N_2H_4 \cdot H_2O$ (100 μL) in DMF (5 mL) was added and the resin agitated for 20 min. The resin was separated by filtration and the cleavage was repeated. Finally, the resin was washed with DMF (3×), and the filtrate was concentrated. CC (DCM/MeOH 40:1, 1% Et_3N) yielded **15** (91 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ = 1.19 (d, *J* = 7.0 Hz, 3 H; CH_3(Ala)), 1.51, 1.54 (2s, 6 H; 2 CH_3(Aib)), 3.13 (q, *J* = 7.0 Hz, 1 H; CH(α)(Ala)), 3.26 (s, 3 H; CH_3 of (N(CH_3)Ph)), 7.41-7.23 (m, 5 arom. H). NH and NH_2 could not be detected. MS (ESI): *m*/*z* (%): 157 (46) oxazolone of $[M–HN(CH_3)Ph+H]^+$, 264 (100) $[M+H]^+$, 286 (6) $[M+Na]^+$, 527 (5) $[2M+H]^+$.

4.6. General Procedures

a) Attachment of the First Amino Acid: All manipulations were carried out under N_2. PAM or HM resin was swollen in THF. After filtration, a solution of $COCl_2$ in toluene (1.9M, 10 equiv.) and THF (ca. 2.5 mL/1 g resin) were added to the resin, which was agitated at r.t. for 2 h, then washed with THF (2×) and DCM (2×). In a separate vial H-AA-O^{t}Bu · HCl (4 equiv.) was dissolved in DIPEA (8 equiv.) and DCM (conc. (AA) = 0.2M). This mixture was added to the resin, whereas possibly occuring ammonium salt was removed by filtration. The resin was agitated at r.t. over night, then washed with DMF (3×) and DCM (3×).

b) Removing the ^{t}Bu Protecting Group: The resin was swollen in DCM. TFA in DCM (1×5 s, 25%; 1×30 min, 50%) and TIPS (5%, in each case) were added and the resin agitated at r.t. Afterwards, the resin was washed with DCM (3×), DMF (2×) and DCM (3×).

c) Coupling with *N*,2,2-Trimethyl-*N*-phenyl-2*H*-azirin-3-amine (2): The resin was swollen in DCM. A solution of **2** (4 equiv.) in DCM (conc. of **2** = 0.2M) was added and the resin agitated at r.t. over night, then washed with DCM (3×). Unconsumed **2** can easily be recovered.

d) Hydrolysis of the Terminal Amide: The resin was swollen in THF. HCl (ca. 3–4 mL/200 mg resin, 3M in THF/H_2O, prepared from conc. HCl and THF) was added and the resin agitated at r.t. over night, then washed with THF (3×), DMF (3×) and DCM (3×).

e) Coupling with H-AA-O^{t}Bu · HCl (method A): The resin was swollen in DCM. PyBOP (4 equiv.) in DCM, then H-AA-O^{t}Bu · HCl (4 equiv.) in DCM and DIPEA (12 equiv.) were added (conc. (AA) = 0.2M) and the resin agitated at r.t. over night, then washed with DCM (2×), DMF (2×) and DCM (3×).

f) Coupling with H-AA-O^{t}Bu · HCl (method B, according to Gausepohl et al.[47]): The resin was swollen in DMF. HOBt (6 equiv.) in DMF, PyBOP (4 equiv.) in DMF, NMM (2.3 equiv.), then H-AA-O^{t}Bu · HCl (4 equiv.) in DMF and NMM (4 equiv.) were added (conc. (AA) = 0.2M). The resin was agitated at r.t. and, after 10 and 20 min, additional NMM (2.3 equiv., each) was added. The resin was agitated at r.t. for 1 h and 5 h for coupling to Aib, resp. The resin was washed with DMF (3×) and DCM (3×).

g) Coupling with H-AA-O^{t}Bu · HCl (method C): The resin was swollen in DCM. PfpOH (4 equiv.) in DCM, PyBOP (4 equiv.) in DCM, then H-AA-O^{t}Bu · HCl (4 equiv.) in DCM and DIPEA (12 equiv.) were added (conc. (AA) = 0.2M). The resin was agitated at r.t. over night, then washed with DCM (2×), DMF (2×) and DCM (3×).

h) Cleavage: The resin was swollen in DCM. HBr in AcOH (33%, 1 mL/100 mg resin) and two drops of water were addeed and the resin agitated for 5 to 6 h. The resin was separated by filtration and washed with AcOH/DCM (1:1, 3×) and MeCN/DCM (1:1, 3×). The solvents were evaporated under reduced pressure and the crude product was purified by means of HPLC. The purified product was lyophilized.

4.7. H-Ala-Aib-Phe-OH (21)

PAM resin (202 mg, 0.125 mmol) was treated according to general procedures a), b), c), d), e) and h) to yield, after purification by means of prep. HPLC and lyophylization, **21** (32 mg, 50%) as a colorless powder.

HM resin (201 mg, 0.197 mmol) was treated according to general procedures a), b), c), d), e) and h) to yield, after precipitation in ether or purification by means of prep. HPLC and lyophylization, **21** (33 mg, 40%) as a colorless powder. HPLC-MS: t_R = 11.6 min, *m/z* (%): 129 (31), 157 (47) oxazolone of $[M–Phe+H]^+$, 251 (28) $[M–Ala+H]^+$, 322 (100) $[M+H]^+$, 344 (8) $[M+Na]^+$. MS (ESI): *m/z* (%): 322 (100) $[M+H]^+$, 344 (60) $[M+Na]^+$. IR (KBr): $\tilde{\nu}$ = 3422*s*, 3575*s*, 3237*s*, 3068*s*, 3034*s*, 2992*s*, 2945*s*, 2617*w*, 1724v*s*, 1671v*s*, 1534v*s*, 1500*s*, 1467*m*, 1457*m*, 1443*m*, 1392*m*, 1369*m*, 1332*w*, 1266*s*, 1201v*s*, 1142v*s*, 1031*m*, 1003*w*, 839*w*, 800*m*, 723*m*, 702*m*, 641*m*, 518*w* cm^{-1}. ^1H NMR (600 MHz, $[D_6]$DMSO, 25°C, TMS): δ = 1.32 (d, *J* = 7.0 Hz, 3 H; CH_3(Ala)), 1.34, 1.35 (2s, 6 H; CH_3(Aib)), 2.95 (dd, *J* (CH_AH_B,CH(α)) = 8.3 Hz, ^2J = 13.7 Hz, 1 H; CH_AH_B(Phe)), 3.08 (dd, *J* (CH_AH_B,CH(α)) = 5.3 Hz, ^2J = 13.7 Hz, 1 H; CH_AH_B(Phe)), 3.82-3.83 (m, 1 H; CH(α)(Ala)), 4.44 (*ddd*, *J* (CH(α),CH_AH_B) = 8.3 Hz, *J* (CH(α),NH) = 8.0 Hz, *J* (CH(α),CH_AH_B) = 5.3 Hz, 1 H; CH(α)(Phe)), 7.18-7.28 (m, 5 arom. H), 7.53 (d, *J* (NH,CH(α)) = 8.0 Hz, 1 H; NH(Phe)), 8.04 (br. s, 3 H; NH_3^+(Ala)), 8.35 (s, 1 H; NH(Aib)), ca. 12.0-13.5 (br. s, 1 H; COOH). ^{13}C NMR (150 MHz, $[D_6]$DMSO, 25°C, TMS): δ = 17.1 (q, CH_3(Ala)), 24.4, 25.0 (2q, 2 CH_3(Aib)), 36.7 (t, CH_2), 48.3 (d, CH(α)(Ala)), 53.5 (d, CH(α)(Phe)), 56.3 (s, C(α)(Aib)), 126.4 (d, arom. CH(*p*)), 128.1 (d, arom. CH(*m*)), 129.3 (d, arom. CH(*o*)), 137.5 (s, arom. C), 168.9 (s, CO(Ala)), 172.7 (s, CO(Phe)), 172.9 (s, CO(Aib)).

4.8. Ac-Ala-Aib-Phe-OMe (26) (Derivatizing 21)

The crude product **21** of a 0.062 mmol batch was dissolved in $(NH_4)_2CO_3$ (2.4 mL, 0.1 M), then Ac_2O/MeOH (6 mL, 1:3) was added and the solution stirred at r.t. for 1 h. After lyophilization, the residue was dissolved in MeOH/H_2O (2 mL, 10:1) and a solution of CH_2N_2 in Et_2O (ca. 2 mL, ca. 1 M) was added . The solution was stirred at r.t., then concentrated in vacuum. HPLC-MS: t_R = 14.8 min, *m/z* (%): 120 (45), 180 (92) [H-Phe-OMe+H]$^+$, 199 (84) oxazolone of [M–(Phe-OMe)+H]$^+$, 265 (100) [M–(Ac-Ala)+H]$^+$, 378 (52) [M+H]$^+$, 400 (71) [M+Na]$^+$. Prep. TLC (DCM/MeOH, 20:1) yielded **26** (9 mg, 38%) as a colorless powder. HPLC-MS: t_R = 14.8 min, *m/z* (%): 120 (37), 180 (100) [H-Phe-OMe+H]$^+$, 199 (63) oxazolone of [M–(Phe-OMe)+H]$^+$, 265 (82) [M–(Ac-Ala)+H]$^+$, 378 (48) [M+H]$^+$, 400 (89) [M+Na]$^+$.

4.9. H-Ala-Aib-Val-Aib-Phe-OH (22)

PAM resin (402 mg, 0.249 mmol) was treated according to general procedure a) and dried in vacuum. The synthesis was continued with half of the resin according to general procedures b), c), d), e), b), c), d), e) and h) to yield, after purification by means of prep. HPLC and lyophylization, **22** (25 mg, 33%) as a colorless powder.

HM resin (501 mg, 0.491 mmol) was treated according to general procedure a) and dried in vacuum. The synthesis was continued with 25% of the resin according to general procedures b), c), d), e), b), c), d), e) and h) to yield, after purification by means of prep. HPLC and lyophylization, **22** (16 mg, 21%) as a colorless powder. HPLC-MS: t_R = 10.4 min, *m/z* (%): 256 (11), 341 (48) oxazolone of [M–Phe+H]$^+$, 506 (100) [M+H]$^+$. MS (ESI): *m/z* (%): 506 (100) [M+H]$^+$, 528 (31) [M+Na]$^+$, 550 (9) [MNa+Na]$^+$. IR (KBr): $\tilde{\nu}$ = 3428*m*, 3308*s*, 3065*s*, 2976*s*, 2940*s*, 2622*w*, 1722*s*, 1668v*s*, 1529v*s*, 1468*m*, 1458*m*, 1389*m*, 1367*m*, 1322*w*, 1263*m*, 1202v*s*, 1139*s*, 1004*w*, 929*w*, 837*w*, 800*w*, 722*m*, 701*w*, 598*w* cm^{-1}. ^{1}H NMR (500 MHz, [D_6]DMSO, 25°C, TMS): δ = 0.81, 0.85 (2d, *J* = 6.8 Hz, 6 H; 2 CH_3(Val)), 1.34 (d, *J* = 6.9 Hz, 3 H; CH_3(Ala)), 1.29, 1.35, 1.38, 1.41 (4s, 12 H; 4 CH_3(Aib)), 2.01 (oct., *J* = 6.8 Hz, 1 H; CH(β)(Val)), 2.94 (dd, *J* (CH_AH_B,CH(α)) = 8.1 Hz, ^2J = 13.8 Hz, 1 H; CH_AH_B(Phe)), 3.03 (dd, *J* (CH_AH_B,CH(α)) = 5.7 Hz, ^2J = 13.8 Hz, 1 H; CH_AH_B(Phe)), 3.85 (q, *J* = 6.9 Hz, 1 H; CH(α)(Ala)), 4.02 (t, *J* = 7.3 Hz, 1 H; CH(α)(Val)), 4.41 (ddd, *J* (CH(α),CH_AH_B) = 8.1 Hz, *J* (CH(α),NH) = 7.9 Hz, *J* (CH(α),CH_AH_B) = 5.7 Hz, 1 H; CH(α)(Phe)), 7.18-7.27 (m, 5 arom. H), 7.30 (d, *J* = 7.7 Hz, 1 H; NH(Val)), 7.48 (d, *J* (NH,CH(α)) = 7.9 Hz, 1 H; NH(Phe)), 7.5-8.5 (br. s, 3 H; NH_3^+(Ala)), 7.91, 8.48 (2s, 2 H; 2 NH(Aib)), ca. 11.0-13.5 (br. s, 1 H; COOH). ^{13}C NMR (125 MHz, [D_6]DMSO, 25°C, TMS): δ = 17.0 (q, CH_3(Ala)), 18.4, 19.3 (2q, 2

CH_3(Val)), 24.3, 24.9, 25.0, 25.1 (4q, 4 CH_3(Aib)), 30.1 (d, CH(β)(Val)), 36.8 (t, CH_2(Phe)), 48.4 (d, CH(α)(Ala)), 53.6 (d, CH(α)(Phe)), 56.1, 56.5 (2s, 2 C(α)(Aib)), 58.4 (d, CH(α)(Val)), 126.4 (d, arom. CH(*p*)), 128.1 (d, arom. CH(*m*)), 129.2 (d, arom. CH(*o*)), 137.5 (s, arom. C), 169.1 (s, CO(Ala)), 170.4 (s, CO(Val)), 172.6 (s, CO(Phe)), 173.3, 173.5 (2s, 2 CO(Aib)).

4.10. H-Ala-Aib-Ala-Gln-Aib-Val-OH (23)

PAM resin (202 mg, 0.125 mmol) was treated according to general procedures a), b), c), d), e), b), f), b), c), d), f) and h) to yield, after purification by means of prep. HPLC and lyophylization, **23** (21 mg, 25%) as a colorless powder. HPLC-MS: t_R = 8.3 min, *m/z* (%): 441 (10) oxazolone of $[M–Val+H]^+$, 558 (100) $[M+H]^+$. MS (ESI): *m/z* (%): 558 (29) $[M+H]^+$, 580 (100) $[M+Na]^+$, 602 (74) $[MNa+Na]^+$. IR (KBr): $\tilde{\nu}$ = 3315*s*, 3065*m*, 2984*m*, 2942*m*, 2614*w*, 1665v*s*, 1533*s*, 1467*m*, 1455*m*, 1424*w*, 1390*m*, 1368*w*, 1332*w*, 1267*m*, 1201*s*, 1140*s*, 1042*w*, 1004*w*, 930*w*, 836*w*, 800*w*, 722*w*, 598*w*, 518*w* cm^{-1}. ^{1}H NMR (500 MHz, [D_6]DMSO, 25°C, TMS): δ = 0.82, 0.85 (2d, *J* = 6.8 Hz, 6 H; 2 CH_3(Val)), 1.23 (d, *J* = 7.0 Hz, 3 H; CH_3(Ala)), 1.36, 1.38 (2s, 6 H; 2 CH_3(Aib)), 1.38 (d, *J* = 6.9 Hz, 3 H; CH_3(Ala)), 1.40 (s, 6 H; 2 CH_3(Aib)), 1.84-1.93 (m, 2 H; CH_2(Gln)), 2.01-2.08 (m, 1 H; CH(β)(Val)), 2.10-2.14 (m, 2 H; CH_2(Gln)), 3.84 (q, *J* = 7.0 Hz, 1 H; CH(α)(Ala)), 4.06-4.10 (m, 2 H; CH(α)(Val), CH(α)(Gln)), 4.15 (quint., *J* = 6.9 Hz,1 H; CH(α)(Ala)), 6.89 (s, 1 H; NH_2(Gln)), 7.12 (d, *J* = 8.5 Hz, 1 H; NH(Val)), 7.40 (s, 1 H; NH_2(Gln)), 7.66 (d, *J* = 6.3 Hz, 1 H; NH(Ala)), ca. 7.7-8.4 (br. s, 3 H; NH_3^+(Ala)), 7.84 (d, *J* = 7.1 Hz, 1 H; NH(Gln)), 7.86 (s, 1 H; NH(Aib)), 8.63 (s, 1 H; NH(Aib)), ca. 11.8-13.1 (br. s, 1 H; COOH). ^{13}C NMR (125 MHz, [D_6]DMSO, 25°C, TMS): δ = 17.0, 17.5 (2q, 2 CH_3(Ala)), 18.0, 19.1, (2q, 2 CH_3(Val)), 24.2, 24.6, 24.9, 25.7 (4q, 4 CH_3(Aib)), 27.4 (t, CH_2(Gln)), 29.9 (d, CH(β)(Val)), 31.8 (t, CH_2(Gln)), 48.3, 48.9 (2d, 2 CH(α)(Ala)), 53.1 (d, CH(α)(Gln)), 56.2, 56.2 (2s, 2 C(α)(Aib)), 57.3 (d, CH(α)(Val)), 169.3 (s, CO(Ala)), 171.0 (s, CO(Gln)), 172.3 (s, CO(Ala)), 172.8 (s, CO(Val)), 173.4, 173.7 (2s, 2 CO(Aib)), 174.2 (s, $CONH_2$).

4.11. H-Leu-Aib-Leu-Aib-Phe-OH (24)

PAM resin (201 mg, 0.125 mmol) was treated according to general procedures a), b), c), d), e), b), c), d), e) and dried in vacuum. One part (14%) was cleaved according to general procedure h) and the crude product analyzed by HPLC-MS. With the other part (86%) a second coupling was performed according to general procedure g). Cleavage according to general procedure h) yielded, after purification by means of prep. HPLC and lyophylization,

24 (15 mg, 20%) as a colorless powder. HPLC-MS: t_R = 12.0 min, *m/z* (%): 397 (15) oxazolone of $[M–Phe+H]^+$, 562 (100) $[M+H]^+$. IR (KBr): $\tilde{\nu}$ = 3423*m*, 3320*s*, 3064*m*, 3034*m*, 2962*s*, 2939*s*, 2874*m*, 1723*sh*, 1668v*s*, 1529v*s*, 1468*m*, 1441*m*, 1388*m*, 1367*m*, 1269*m*, 1202v*s*, 1140*s*, 1081*w*, 1031*w*, 941*w*, 878*w*, 837*w*, 800*w*, 722*w*, 700*w*, 598*w*, 518*w*, 490*w* cm^{-1}. ^1H NMR (600 MHz, $[D_6]$DMSO, 25°C, TMS): δ = 0.83, 0.86, 0.90, 0.92 (4d, *J* = 6.3 Hz, 12 H; 4 CH_3(Leu)), 1.30, 1.36, 1.38, 1.39 (4s, 12 H; 4 CH_3(Aib)), 1.40-1.65 (m, 6 H; 2 CH_2(Leu), 2 CH(γ)(Leu)), 2.95 (dd, *J* (CH_AH_B,CH(α)) = 8.3 Hz, ^2J = 13.8 Hz, 1 H; CH_AH_B(Phe)), 3.04 (dd, *J* (CH_AH_B,CH(α)) = 5.4 Hz, ^2J = 13.8 Hz, 1 H; CH_AH_B(Phe)), 3.77 (br. s, 1 H; CH(α)(Leu)), 4.15 (m, 1 H; CH(α)(Leu)), 5.41 (ddd, *J* (CH(α),CH_AH_B) = 8.3 Hz, *J* (CH(α),NH) = 7.8 Hz, *J* (CH(α),CH_AH_B) = 5.4 Hz, 1 H; CH(α)(Phe)), 7.18-7.26 (m, 5 arom. H), 7.51 (d, *J* (NH,CH(α)) = 7.8 Hz, 1 H; NH(Phe)), 7.59 (d, *J* = 7.4 Hz, 1 H, NH(Leu)), 7.80 (s, 1 H; NH(Aib)), 8.08 (br. s, 3 H; NH_3^+(Leu)), 8.58 (s, 1 H, NH(Aib)), ca. 12.4-13.0 (br. s, 1 H; COOH). ^{13}C NMR (150 MHz, $[D_6]$DMSO, 25°C, TMS): δ = 21.5, 21.9, 22.5, 23.1 (4q, 4 CH_3(Leu)), 23.6, 24.0 (2d, 2 CH(γ)(Leu)), 24.0, 24.2, 25.0, 25.5 (4q, 4 CH_3(Aib)), 36.7 (t, CH_2(Phe)), 39.5, 39.7 (2t, 2 CH_2(Leu)), 51.1, 51.8 (2d, 2 CH(α)(Leu)), 53.6 (d, CH(α)(Phe)), 55.9, 56.4 (2s, 2 C(α)(Aib)), 126.3 (d, arom. CH(*p*)), 128.0 (d, arom. CH(*m*)), 129.1 (d, arom. CH(*o*)), 137.5 (s, arom. C), 168.4, 171.3 (2s, 2 CO(Leu)), 172.6 (s, CO(Phe)), 173.3, 173.8 (2s, 2 CO(Aib)).

4.12. H-Val-Aib-Gly-Aib-Ala-OH (25)

PAM resin (200 mg, 0.124 mmol) was treated according to general procedures a), b), c), d), e), b), c), d), e) and h) to yield, after purification by means of prep. HPLC and lyophylization, **25** (20 mg, 31%) as a colorless powder. HPLC-MS: t_R = 9.8 min, *m/z* (%): 327 (51) oxazolone of $[M–Ala+H]^+$, 416 (100) $[M+H]^+$. IR (KBr): $\tilde{\nu}$ = 3315*s*, 3066*s*, 2985*s*, 2943*s*, 2642*w*, 1667v*s*, 1537v*s*, 1468*m*, 1387*m*, 1367*m*, 1335*w*, 1296*m*, 1248*m*, 1202v*s*, 1140*s*, 1018*w*, 980*w*, 946*w*, 837*w*, 800*w*, 722*m*, 662*w*, 598*w*, 562*w*, 518*w* cm^{-1}. ^1H NMR (500 MHz, $[D_6]$DMSO, 25°C, TMS): δ = 0.94, 0.95 (2d, *J* = 6.7 Hz, 6 H; 2 CH_3(Val)), 1.27 (d, *J* = 7.3 Hz, 3 H; CH_3(Ala)), 1.38, 1.39, 1.41 (3s, 12 H; 4 CH_3(Aib)), 2.10 (oct., *J* = 6.7 Hz, 1 H; CH(β)(Val)), 3.52 (dd, *J* (NH,CH_AH_B) = 5.6 Hz, ^2J = –16.3 Hz, 1 H; CH_AH_B(Gly)), 3.60 (d, *J* = 6.2 Hz, 1 H; CH(α)(Val)), 3.65 (dd, *J* (CH_AH_B,NH) = 5.7 Hz, ^2J = –16.3 Hz, 1 H; CH_AH_B(Gly)), 4.16 (quint., *J* = 7.3 Hz, 1 H; CH(α)(Ala)), 7.47 (d, *J* = 7.3 Hz, 1 H; NH(Ala)), ca. 7.6-8.5 (br. s, 3 H; NH_3^+(Val)), 7.70 (s, 1 H; NH(Aib)), 8.01 (dd, *J* (NH,CH_AH_B) = 5.7 Hz, *J* (NH,CH_AH_B) = 5.6 Hz, 1 H; NH(Gly)), 8.70 (s, 1 H; NH(Aib)), ca. 11.8-13.0 (br. s, 1 H; COOH). ^{13}C NMR (125 MHz, $[D_6]$DMSO, 25°C, TMS): δ = 17.0 (q, CH_3(Ala)), 17.6, 18.4,

(2q, 2 CH_3(Val)), 23.7, 23.9, 25.8, 25.8 (4q, 4 CH_3(Aib)), 29.6 (d, CH(β)(Val)), 43.4 (t, CH_2(α)(Gly)), 47.7 (d, CH(α)(Ala)), 55.9, 56.3 (2s, 2 C(α)(Aib)), 57.6 (d, CH(α)(Val)), 167.8 (s, CO(Val)), 168.3 (s, CO(Gly)), 173.7 (s, CO(Aib)), 174.0 (s, CO(Ala)), 174.1 (s, CO(Aib)).

References

[1] R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149 – 2154.

[2] G. B. Fields, R. L. Noble, *Int. J. Pept. Protein Res.* **1990**, *35*, 161 – 214.

[3] P. Lloyd-Williams, F. Alberico, E. Giralt, *Tetrahedron* **1993**, *49*, 11065 – 11133.

[4] W. G. Gutheil, Q. Xu, *Chem. Pharm. Bull.* **2002**, *50*, 688 – 691.

[5] R. L. Letsinger, M. J. Kornet, *J. Am. Chem. Soc.* **1963**, *85*, 3045 – 3046.

[6] A. M. Felix, R. B. Merrifield, *J. Am. Chem. Soc.* **1970**, *92*, 1385 – 1391.

[7] B. Henkel, L. Zhang, E. Bayer, *Liebigs Ann./Recueil* **1997**, 2161 – 2168.

[8] A. Johansson, E. Åkerblom, K. Ersmark, G. Lindeberg, A. Hallberg, *J. Comb. Chem.* **2000**, *2*, 496 – 507.

[9] F. Bordusa, D. Ullmann, H.-D. Jakubke, *Angew. Chem., Int. Ed.* **1997**, *36*, 1099 – 1101.

[10] H. Wenschuh, M. Beyermann, E. Krause, M. Brudel, R. Winter, M. Schümann, L. A. Carpino, M. Bienert, *J. Org. Chem.* **1994**, *59*, 3275 – 3280.

[11] H. Wenschuh, M. Beyermann, H. Haber, J. K. Seydel, E. Krause, M. Bienert, L. A. Carpino, A. El-Faham, F. Alberico, *J. Org. Chem.* **1995**, *60*, 405 – 410.

[12] M. Meldal, M. A. Juliano, A. M. Jansson, *Tetrahedron Lett.* **1997**, *38*, 2531 – 2534.

[13] W. F. DeGrado, *Adv. Prot. Chem* **1988**, *39*, 51 – 124.

[14] J. Rizo, L. M. Gierasch, *Annu. Rev. Biochem.* **1992**, *61*, 387 – 416.

[15] V. J. Hruby, F. Al-Obeidi, W. Kazmierski, *Biochem. J.* **1990**, *268*, 249 – 262.

[16] M. Mutter, *Angew. Chem., Int. Ed.* **1985**, *24*, 639 – 653.

[17] W. Mayr, G. Jung, J. Strähle, *Liebigs Ann. Chem.* **1980**, 715 – 724.

[18] U. Slomczynska, D. D. Beusen, J. Zabrocki, K. Kociolek, A. Redlinski, F. Reusser, W. C. Hutton, M. T. Leplawy, G. R. Marshall, *J. Am. Chem. Soc.* **1992**, *114*, 4095 – 4106.

[19] S. Vijayalakshmi, R. Balaji Rao, I. L. Karle, P. Balaram, *Biopolymers* **2000**, *53*, 84 – 98.

[20] N. Lancelot, K. Elbayed, J. Raya, M. Piotto, J.-P. Briand, F. Formaggio, C. Toniolo, A. Bianco, *Chem. Eur. J.* **2003**, *9*, 1317 – 1323.

[21] H. Heimgartner, *Angew. Chem., Int. Ed.* **1991**, *30*, 238 – 264.

[22] J. M. Humphrey, A. R. Chamberlin, *Chem. Rev.* **1997**, *97*, 2243 – 2266.

[23] P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1990**, *73*, 13 – 24.

[24] R. T. N. Luykx, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2003**, *86*, 4093 – 4111.

[25] N. Pradeille, H. Heimgartner, *J. Pept. Sci.* **2003**, *9*, 827 – 837.

[26] S. Stamm, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2003**, *86*, 1371 – 1396.

[27] J. Lehmann, A. Linden, H. Heimgartner, *Tetrahedron* **1998**, *54*, 8721 – 8736.

[28] C. B. Bucher, H. Heimgartner, *Helv. Chim. Acta* **1996**, *79*, 1903 – 1915.

[29] After PyBOP mediated coupling of carboxylpolystyrene and H-Ala-O^{t}Bu, the ^{t}Bu ester was hydrolyzed in TFA/DCM. The resin was treated with *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**2**), then the terminal amide was hydrolyzed with 3M HCl and finally PyBOP mediated coupling with H-Phe-O^{t}Bu yielded the resin-bound tripeptide Ala-Aib-Phe-O^{t}Bu. After every reaction, the resin was examined by ATR-FT-IR spectroscopy. Acidic hydrolysis (propionic acid/HCl, 130°C) of the resin-bound tripeptide gave Ala, Aib and Phe, which were detected by ESI-MS.

[30] S. R. Chhabra, A. N. Khan, B. W. Bycroft, *Tetrahedron Lett.* **1998**, *39*, 3585 – 3588.

[31] R. L. Letsinger, M. J. Kornet, V. Mahadevan, D. M. Jerina, *J. Am. Chem. Soc.* **1964**, 5163 – 5165.

[32] D. J. Burdick, M. E. Struble, J. P. Burnier, *Tetrahedron Lett.* **1993**, *34*, 2589 – 2592.

[33] J. R. Hauske, P. Dorff, *Tetrahedron Lett.* **1995**, *36*, 1589 – 1592.

[34] B. Raju, T. P. Kogan, *Tetrahedron Lett.* **1997**, *38*, 3373 – 3376.

[35] F. Wang, J. R. Hauske, *Tetrahedron Lett.* **1997**, *38*, 6529 – 6532.

[36] B. A. Dressman, L. A. Spangle, S. W. Kaldor, *Tetrahedron Lett.* **1996**, *37*, 937 – 940.

[37] C. Y. Ho, M. J. Kukla, *Tetrahedron Lett.* **1997**, *38*, 2799 – 2802.

[38] R. Léger, R. Yen, M. W. She, V. J. Lee, S. J. Hecker, *Tetrahedron Lett.* **1998**, *39*, 4171 – 4174.

[39] W. J. N. Meester, F. P. J. T. Rutjes, P. H. H. Hermkens, H. Hiemstra, *Tetrahedron Lett.* **1999**, *40*, 1601 – 1604.

[40] Some loss of peptide was observed during deprotection of ^{t}Bu esters with TFA on hydroxymethyl polystyrene resin. When using hydroxyethylpolystyrene resin no cleavage from the support was achieved with either trifluoromethanesulfonic acid or HBr in acetic acid.

[41] Performed by C.A.T. GmbH & Co., Tübingen, Germany.

[42] *Interchim Uptisphere HDO C18*, 120 Å, 3 μm, 200 × 2.0 mm.

[43] The crude product was dissolved in aq. $(NH_4)_2CO_3$ (0.1 M), then Ac_2O/MeOH (1:3) was added and the solution stirred at r.t. for 1 h. After lyophilization, the residue was dissolved in MeOH/H_2O (10:1) and a solution of CH_2N_2 in Et_2O was added. The solution was stirred at r.t. and then concentrated in vacuum.

[44] H. Brückner, ed., 'Special Issue: Peptaibols/Peptaibiotics', *J. Pept. Sci.* **2003**, *9*, 663 – 837.

[45] H. Wenschuh, M. Beyermann, E. Krause, L. A. Carpino, M. Bienert, Innovation Perspect. Solid Phase Synth. Collect. Pap., Int. Symp., 3rd (1994), Meeting Date 1993, 697 – 700.

[46] H. Wenschuh, M. Beyermann, S. Rothemund, L. A. Carpino, M. Bienert, *Tetrahedron Lett.* **1995**, *36*, 1247 – 1250.

[47] H. Gausepohl, M. Kraft, R. W. Frank, *Int. J. Pept. Protein Res.* **1989**, *34*, 287 – 294.

[48] Nevertheless a minor product (m/z = 658, $[M+Val+H]^+$) was detected. Probably the amide group in the side chain of Gln hydrolyzed partially in 3 M HCl, so that two molecules of Val could add in the subsequent coupling reaction.

[49] M. Crisma, A. Barazza, F. Formaggio, B. Kaptein, Q. Broxtermann, J. Kamphuis, C. Toniolo, *Tetrahedron* **2001**, *57*, 2813 – 2825.

[50] A. Jaworski, H. Brückner, *J. Pept. Sci.* **2001**, *7*, 433 – 447.

[51] J. M. Villalgordo, H. Heimgartner, *Tetrahedron* **1993**, *49*, 7215 – 7222.

[52] J. M. Villalgordo, H. Heimgartner, *Helv. Chim. Acta* **1993**, *76*, 2830 – 2837.

[53] F. C. Westall, J. Scotchler, A. B. Robinson, *J. Org. Chem.* **1972**, *37*, 3363 – 3365.

[54] Fragment of Phe (shown by MS^3).

[55] S. R. Chhabra, H. Parekh, A. N. Khan, B. W. Bycroft, B. Kellam, *Tetrahedron Lett.* **2001**, *42*, 2189 – 2192.

5. The 'Azirine/Oxazolone Method' on Solid-Phase: Introduction of Various α,α-Disubstituted α-Amino Acids[1]

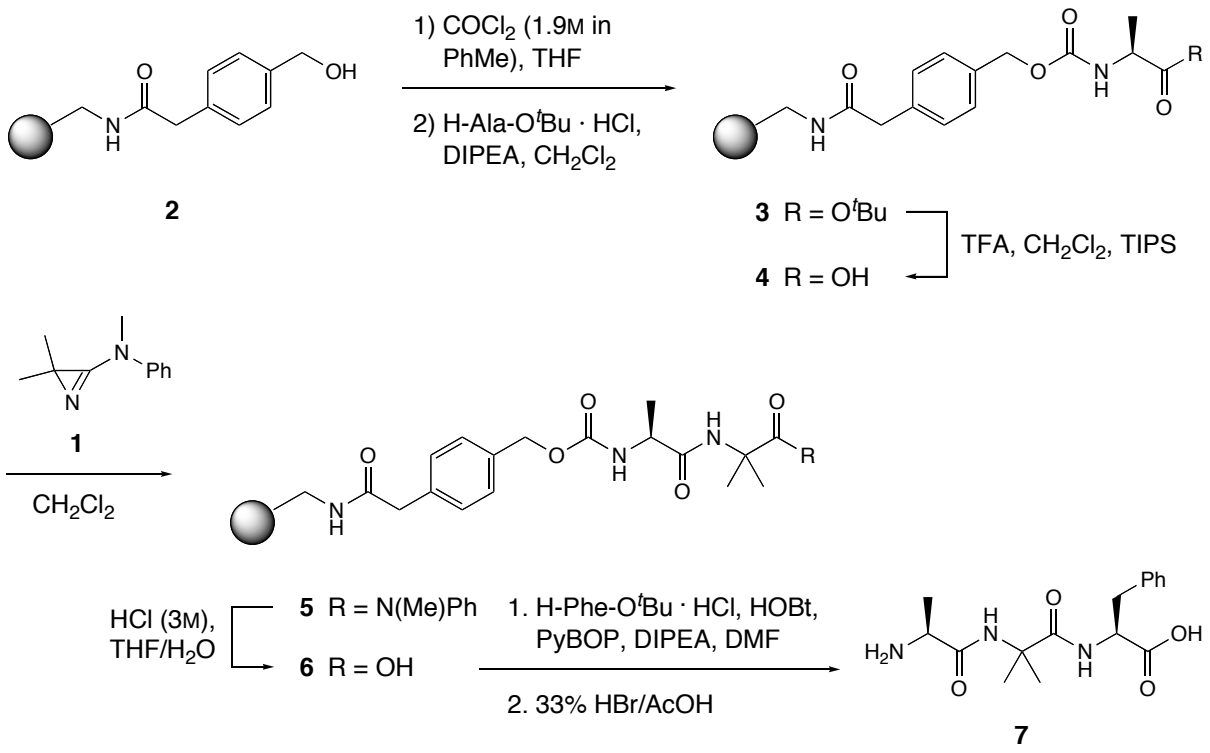
Peptides containing various α,α-disubstituted α-amino acids, such as α-aminoisobutyric acid (Aib), 1-aminocyclopentane-1-carboxylic acid, α-methylphenylalanine, and 4-aminotetrahydro-2*H*-pyran-4-carboxylic acid have been synthesized from the N- to the C-terminus by means of the 'azirine/oxazolone method' under solid-phase conditions. In this convenient method for the synthesis of sterically demanding peptides on solid-phase, 2*H*-azirin-3-amines are used to introduce the α,α-disubstituted α-amino acids without the need of additional reagents. Furthermore, the synthesis of poly-Aib sequences has been explored.

1. Introduction

Due to the restrictions in their conformational freedom, α,α-disubstituted α-amino acid containing peptides form stabilized secondary structures, such as β-turns and helices [1–4]. One useful method for the introduction of α,α-disubstituted α-amino acids into peptides is the 'azirine/oxazolone method' [5–7]. Thus, the reaction of 2*H*-azirin-3-amines, *e.g.* the Aib synthon **1**, with an amino or peptide acid proceeds smoothly and in high yield. The terminal amide bond of the resulting peptide amide can be hydrolyzed selectively to give the extended peptide acid. In solution-phase chemistry, the 'azirine/oxazolone method' has proven to be successful for the introduction of a multitude of sterically demanding α,α-disubstituted α-amino acids into peptides, and it has found application in the synthesis of some antibiotic peptaibols or segments thereof [8–15].

Since solid-phase synthesis offers rapid access to peptides without the need for the isolation of the sometimes cumbersome peptide acid intermediates, we adapted the 'azirine/oxazolone method' to solid-phase conditions (*Scheme 1*) [16].

[1] S. Stamm, A. Linden, H. Heimgartner, *Helv. Chim. Acta.* **2006**, *89*, 1.

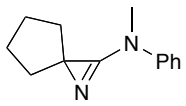


Scheme 1. DIPEA = ethyldiisopropylamine; HOBt: 1-hydroxybenzotriazole; PyBOP = (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TFA = trifluoroacetic acid; TIPS = triisopropylsilane.

In this convenient method for the synthesis of sterically demanding peptides on solid-phase, the first amino acid was attached through a carbamate linker to a [4-(hydroxymethyl)phenyl]acetamidomethyl (PAM) polystyrene resin (**2**). Deprotection of the ^{t}Bu ester **3** with TFA afforded resin **4**, which was treated with a solution of *N*,2,2-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**1**). Unconsumed **1** could easily be recovered and reused. Selective hydrolysis of the terminal amide with 3M HCl in THF/H_2O afforded peptide acid resin **6**. Further extension of the peptide chain could be achieved either with a ^{t}Bu ester protected amino acid and a coupling reagent, *e.g*. PyBOP, or with **1**. Cleavage from the resin was achieved with HBr (33%) in acetic acid to give the tripeptide **7**.

It was of interest to ascertain if this method is restricted to the α-aminoisobutyric acid (Aib) synthon **1**, or if it can be extended to other 2*H*-azirin-3-amines. Herein we report the use of the 1-aminocyclopentane-1-carboxylic acid (Acp) synthon **8**, the 4-aminotetrahydro-2*H*-pyran-4-carboxylic acid (Thp) synthon **9**, and the α-methylphenylalanine (Phe(2Me)) synthon

10 in peptide synthesis using the 'azirine/oxazolone method' under solid-phase conditions. Furthermore, a limitation of the method in the synthesis of poly-Aib sequences is revealed.



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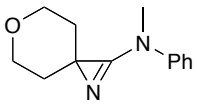

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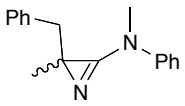

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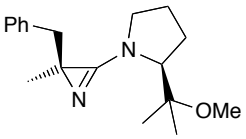

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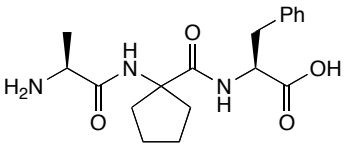
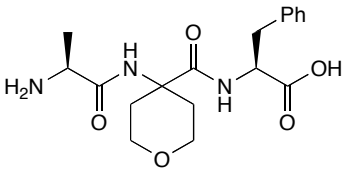
2. Results and Discussion

In analogy to the model peptide H-Ala-Aib-Phe-OH, which had been used to establish the viability of the 'azirine/oxazolone method' under solid-phase conditions, the tripeptides H-Ala-Acp-Phe-OH (**11a**) and H-Ala-Thp-Phe-OH (**11b**) were synthesized on solid-phase in 37% and 38% yield (after prep. HPLC, based on resin loading), respectively (see *Scheme 1*; instead of **1**, the Acp and Thp synthons **8** and **9** were used; *Table 1*). Both α,α-disubstituted residues were introduced by the 'azirine/oxazolone method', and Phe with PyBOP as the coupling reagent.



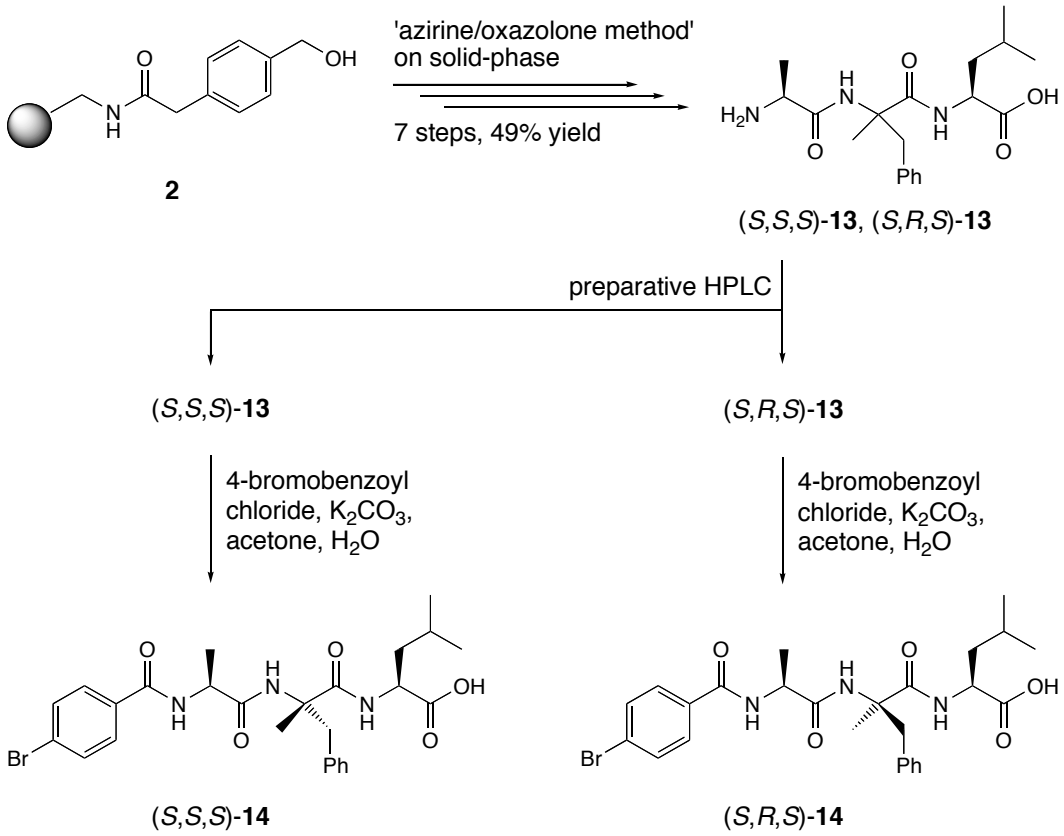
11a



11b

When (*S*)-1-[(*S*)-2-benzyl-2-methyl-2*H*-azirin-3-yl]-2-(1-methoxy-1-methylethyl) pyrrolidine (**12**) was used as an optically pure Phe(2Me) synthon, the syntheses of H-Ala-Phe(2Me)-Phe-OH and H-Ala-Phe(2Me)-Leu-OH failed, although this 2*H*-azirin-3-amine has been used successfully in solution-phase chemistry. *N*-Methyl-*N*-phenyl-2*H*-azirin-3-amines belong to the most reactive 2*H*-azirin-3-amines. Therefore, the racemic Phe(2Me)-synthon **10** was used in a second, successful attempt to synthesize the tripeptide H-Ala-Phe(2Me)-Leu-OH (**13**) as a mixture of two diastereoisomers. The diastereoisomers (*S*,*S*,*S*)-**13** and (*S*,*R*,*S*)-**13** were separated by means of preparative HPLC, which gave the two isomers in a 1:1 ratio in 49% yield.

X-ray crystallography would have allowed the configuration of the C(α)-center of the Phe(2Me) residue to be determined, but all attempts to crystallize at least one of the two diastereoisomeric tripeptides **13** failed. Therefore, (*S*,*S*,*S*)-**13** and (*S*,*R*,*S*)-**13** were derivatized with 4-bromobenzoyl chloride (*Scheme 2*). Crystals, suitable for an X-ray crystal-structure determination were obtained from (*S*,*S*,*S*)-**14** (*Fig. 1*) and the absolute configuration of the molecule was determined independently by the diffraction experiment. This confirmed the (*S*)-configurations of the alanine and leucine residues and revealed the (*S*)-configuration of the Phe(2Me) residue. The knowledge of the absolute configuration of (*S*,*S*,*S*)-**14** allowed the absolute configurations of the primarily isolated tripeptides (*S*,*S*,*S*)-**13** and (*S*,*R*,*S*)-**13** to be assigned.



Scheme 2

The asymmetric unit in the structure of (*S*,*S*,*S*)-**14** contains two symmetry-independent peptide and two MeOH molecules. The two peptide molecules have very similar conformations and differ primarily in the orientation of the plane of the bromophenyl ring. Each peptide molecule is involved in one intramolecular and four intermolecular H-bonds.

The amide group closest to the carboxy group in each peptide molecule forms an intramolecular H-bond with the amide O-atom adjacent to the bromophenyl moiety thereby stabilizing a β-turn. Each of these interactions has a graph set motif [18] of S(10). The OH-group in each peptide molecule forms an intermolecular H-bond with the O-atom of a neighbouring MeOH molecule. In turn, each of these MeOH molecules forms an intermolecular H-bond to one of the symmetry-independent peptide molecules. These interactions link the peptide and MeOH molecules alternately into extended chains which run parallel to the [0 1 0] direction in the sequence ···peptideA···MeOH2···peptideB···MeOH1··· peptideA···. The quaternary graph set motif that describes this sequence is $C_4^4(26)$. The amide group closest to the bromophenyl moiety in peptide molecule A forms an intermolecular H-bond with the carboxylate carbonyl O-atom of a neighbouring molecule B. In turn, molecule B interacts in an identical fashion with another molecule A. These interactions link peptide molecules A and B alternately into extended chains, which run parallel to the [0 -1 1] direction and can be described by a binary graph set motif of $C_2^2(22)$. The central amide group in peptide molecule A forms an intermolecular H-bond with the carbonyl O-atom of the amide group closest to the carboxylic acid end of a neighbouring molecule A. This interaction links the peptide molecules A into centrosymmetric dimers and can be described by a graph set motif of $R_2^2(10)$. The peptide molecules B display identical interactions that also link the molecules into centrosymmetric dimers. The combination of all intermolecular H-bonding interactions links the peptide and MeOH molecules into extended two-dimensional networks which lie parallel to the (1 0 0) plane.

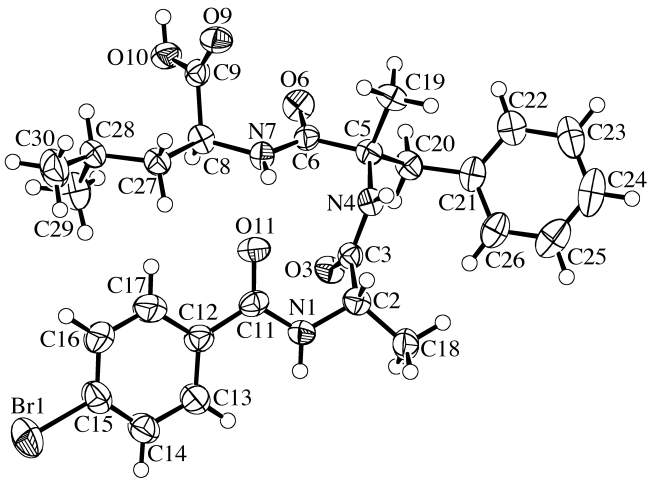


Fig. 1. *ORTEP Plot* [17] *of the molecular structure of one of the two symmetry-independent molecules of* (*S*,*S*,*S*)-**14** (50% probability ellipsoids; arbitrary numbering of atoms; the MeOH molecules are not shown).

Some longer model peptides containing up to three α,α-disubstituted residues were synthesized successfully on solid-phase (*Table 1*). The pentapeptides H-Val-Thp-Gly-Acp-Ala-OH (**15**) and H-Ala-Thp-Val-Thp-Phe-OH (**16**) were obtained in 23% and 16% yield, respectively. All α,α-disubstituted residues were introduced by the 'azirine/oxazolne method', while the couplings of the other amino acids were introduced by using PyBOP as the coupling reagent. The ^{1}H-NMR spectrum of **15** showed partial doubling of the signals. The contribution of the minor signals is *ca.* 20%. Unexpectedly, while increasing the temperature in the NMR experiment, the chemical shifts of the minor and major NH signals did not coalesce or converge. The convergence of the NH signals with increasing temperature would have been strong evidence for the existence of conformers and not diastereoisomers. To rule out the presence of diastereoisomers, however, an amino acid analysis was performed [19]: in order to determine the extent of racemization/epimerization of the C(α) center(s), the pentapeptide was hydrolyzed and the amino acids were analyzed by capillary gas chromatography with enantiomer labelling. The results showed that alanine and valine had racemized by 0.4% and 0.8%, respectively. Thus, the doubling of the signals in the ^{1}H-NMR was caused by different conformers and not by diastereoisomers. Furthermore, this analysis shows that, although the peptide was synthesized from the N- to the C-terminus, the product was obtained with an acceptably low degree of racemization.

Table 1. *Synthesized Peptides Containing Aib, Acp, Thp and Phe(2Me) Residues.*

Sequence	Yield [%] [a])
H-Ala-Acp-Phe-OH (**11a**)	37
H-Ala-Thp-Phe-OH (**11b**)	38
H-Ala-Phe(2Me)-Leu-OH (**13**)	49
H-Val-Thp-Gly-Acp-Ala-OH (**15**)	23
H-Ala-Thp-Val-Thp-Phe-OH (**16**)	16
H-Ala-Aib-Val-Acp-Gly-Thp-Leu-OH (**17**)	21
H-Ala-Aib-Val-Acp-Phe-Thp-Leu-OH (**18**)	13

[a]) Yield of product isolated after HPLC purification, based on resin loading.

The heptapeptides H-Ala-Aib-Val-Acp-Gly-Thp-Leu-OH (**17**) and H-Ala-Aib-Val-Acp-Phe-Thp-Leu-OH (**18**) were synthesized analogously on solid-phase with yields of 21% and 13%, respectively. The two peptides only differ in one amino acid (Gly(4) → Phe(4)). While the coupling of the α,α-disubstituted α-amino acid is a difficult step (which has been solved

by using 2*H*-azirin-3-amines), the following coupling can be difficult too, so we assume that this might be the reason for the noticeably lower yield of **18** compared with **17**.

Peptides containing α,α-disubstituted α-amino acids stabilize or even promote secondary structures, such as helices or β-turns. Therefore, poly-Aib motifs with an accumulation of helix-stabilizing residues are of some interest. The repeated coupling of 2*H*-azirin-3-amines in solution is an efficient method for the preparation of this type of sterically highly congested oligopeptides [20] [21]. The tripeptide H-$(Aib)_3$-OH (**19**) was synthesized using the 'azirine/oxazolone method' on solid-phase in 33% yield (*Table 2*), but the preparation of H-$(Aib)_4$-OH failed. A similar result was obtained in the extension from H-Ala-$(Aib)_2$-OH (**20**) to H-Ala-$(Aib)_3$-OH (**21**). While **20** could be prepared in 41% yield, **21** was obtained in a conspicuously lower yield (*ca.* 12%, not pure; additionally, 35% of **20** were obtained). The introduction of the fourth amino acid in H-Ala-Aib-Aib-Val-OH with conventional coupling by using PyBOP as the coupling reagent was also in vain. All attempts to improve the introduction of the fourth amino acid, such as performing the reaction in different solvents (CH_2Cl_2, THF, DMF, PhMe, H_2O), raising the temperature to 50° or using a *Tentagel* resin were not effective. Since the most probable reason for the failure is aggregation, we also performed the reaction by using the 'magic mixture' [22] (DCM/DMF/NMP (1:1:1), *triton X-100*, ethylenecarbonate (2M)) and in $CHCl_3$/hexafluoroisopropanol (1:1), but no improvement could be achieved.

Table 2. *Synthesized Peptides Containing Poly-Aib Motifs.*

Sequence	Yield [%] [a])
H-Aib-Aib-Aib-OH (**19**)	33
H-Ala-Aib-Aib-OH (**20**)	41
H-Ala-Aib-Aib-Aib-OH (**21**)	12 [b])
H-Ala-Val-Aib-Aib-Aib-OH (**22**)	16
H-Ala-Val-Phe-Aib-Aib-Leu-OH (**23**)	6
H-Ala-Val-Phe-Aib-Aib-Aib-Leu-OH (**24**)	9

[a]) Yield of product isolated after HPLC purification, based on resin loading.

[b]) not pure

A slight improvement was observed if proteinogenic α-amino acids were introduced prior to the poly-Aib motif, *e.g.*, H-Ala-Val-Aib-Aib-Aib-OH (**22**) was prepared in 16% yield. Furthermore, the poly-Aib containing peptides H-Ala-Val-Phe-Aib-Aib-Leu-OH (**23**) and H-Ala-Val-Phe-Aib-Aib-Aib-Leu-OH (**24**) were synthesized, although in low yield (6% and 9%, respectively). All Aib residues were introduced by the 'azirine/oxazolone method', while all other amino acids were introduced by using PyBOP as the coupling reagent.

3. Conclusions

Peptide synthesis using the 'azirine/oxazolone method' on solid-phase was carried out from the N- to the C-terminus. 2*H*-Azirin-3-amines were used to introduce α,α-disubstituted α-amino acids into the peptides without the need for further reagents. It was shown that the method is not limited to the Aib synthon **1**, and it was extended successfully to the 1-aminocyclopentane-1-carboxylic acid (Acp) synthon **8**, the 4-aminotetrahydro-2*H*-pyran-4-carboxylic acid (Thp) synthon **9** and the α-methylphenylalanine (Phe(2Me)) synthon **10**. Peptides with up to seven residues, of which three are α,α-disubstituted α-amino acids, have been prepared. In contrast, the synthesis of peptides containing the poly-Aib motif was not successful, most probably due to aggregation.

Experimental Part

1. *General.* See [16], except analytic HPLC-MS: The system consists of a *Rheos 2000* pump, a *Rheos CPS-LC* degasser (*Flux Instruments*, Basel, Switzerland) and a *Thermo Finnigan Surveyor* photo-diode array detector (*Thermo Finnigan*, San Jose, CA, USA). The HPLC-system is equipped with a *HTS PAL* autosampler (*CTC Analytics*, Zwingen, Switzerland) and connected to a *Thermo Finnigan MSQ* linear quadrupole instrument. Method A: *Interchim Uptisphere C18-NEC*, 120 Å, 3 μm, 50 × 2.0 mm column (*Interchim*, Montluçon, France); eluents: A = H_2O, B = MeCN, C = HCOOH (1%) in H_2O; flow rate: 0.2 ml/min, gradient (A:B:C): 0.0–10.0 min: 85:5:10–75:15:10. Method B: *Interchim Uptisphere C18-ODB*, 120 Å, 3 μm, 50 × 2.0 mm column; eluents: A = H_2O, B = MeCN, C = HCOOH (1%) in H_2O; flow rate: 0.2 ml/min, gradient (A:B:C): 0.0–15.0 min: 87:3:10–40:50:10. *N*,2,2-Trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**1**), *N*-methyl-*N*-phenyl-1-azaspiro[2.4]hept-1-

en-2-amine (**8**), *N*-methyl-*N*-phenyl-6-oxa-1-azaspiro[2.5]oct-1-en-2-amine (**9**), and 2-benzyl-*N*,2-dimethyl-*N*-phenyl-2*H*-azirin-3-amine (**10**) were synthesized by *Villalgordo* and *Heimgartner's* method [23–25]. (*S*)-1-[(*S*)-2-benzy1-2-methy1-2*H*-azirin-3-y1]-2-(1-methoxy-1-methylethyl)pyrrolidine (**12**) was synthesized according to [26]. In the NMR data, the integer *n* in Xaa^n corresponds to the position of the amino acid within the peptide, but is only given if the amino acid was present more than once in the peptide and if the NMR signal could be assigned unambigously. In some ^{1}H-NMR spectra, the broad COOH signal could not be detected. In the ^{1}H- and ^{13}C-NMR spectra of **15**, the descriptor (w) means the weaker, (s) the stronger signal of the two conformers observed.

2. *Abbreviations.* Aib: α-aminoisobutyric acid; CC: column chromatography; Acp: 1-aminocyclopentane-1-carboxylic acid; DCM: dichloromethane; DIPEA: *N*,*N*-diisopropylethylamine; HOBt: 1-hydroxybenzotriazole; PAM: [4-(hydroxymethyl)phenyl]acetamidomethyl; Phe(2Me): 2-amino-2-methyl-3-phenylpropanoic acid; PyBOP: (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; Thp: 4-aminotetrahydro-2*H*-pyran-4-carboxylic acid; TIPS: triisopropylsilane.

3. *General Procedures (GP1–GP6). GP1*: *Attachment of the First Amino Acid.* All manipulations were carried out under N_2. PAM resin was swollen in THF. After filtration, a soln. of $COCl_2$ in toluene (1.9M, 10 equiv.) and THF (*ca.* 2.5 ml/1 g resin) were added to the resin, which was agitated at r.t. for 2 h, then washed with THF (2×) and DCM (2×). In a separate vial, H-Xaa-O^{t}Bu · HCl (4 equiv.) was dissolved in DIPEA (8 equiv.) and DCM (conc. of H-Xaa-O^{t}Bu · HCl = 0.2 M). This mixture was added to the resin, and any ammonium salt that occured was removed by filtration. The resin was agitated at r.t. overnight, then washed with DMF (3×) and DCM (3×).

GP2: *Removing the ^{t}Bu Protecting Group.* The resin was swollen in DCM. TFA in DCM (1×5 s, 25%; 1×30 min, 50%) and TIPS (5%, in each case) were added and the resin agitated at r.t. Afterwards, the resin was washed with DCM (3×), DMF (2×) and DCM (3×).

GP3: *Coupling with 2*H*-azirin-3-amines* **1**, **8**, **9**, **10**. The resin was swollen in DCM. A soln. of 2*H*-azirin-3-amine (4 equiv.) in DCM (conc. of 2*H*-azirin-3-amine = 0.2M) was added and the resin agitated at r.t. overnight, then washed with DCM (3×). Unconsumed 2*H*-azirin-3-amine can easily be recovered.

GP4: *Hydrolysis of the Terminal Amide.* The resin was swollen in THF. Aq. HCl (*ca.* 3-4 ml/200 mg resin, 3M in THF/H_2O, prepared from conc. HCl and THF) was added and the resin agitated at r.t. overnight, then washed with THF (3×), DMF (3×) and DCM (3×).

GP5: *Coupling with H-Xaa-O^{t}Bu · HCl.* The resin was swollen in DMF. HOBt (6 equiv.) in DMF, PyBOP (4 equiv.) in DMF, H-Xaa-O^{t}Bu · HCl (4 equiv.) in DMF and DIPEA (12 equiv.) was added (conc. of H-Xaa-O^{t}Bu · HCl = 0.2M), and the resin agitated at r.t. overnight, then washed with DMF (3×) and DCM (3×).

GP6: *Cleavage*. The resin was swollen in DCM. HBr in AcOH (33%, 1 ml/100 mg resin), two drops of H_2O were added and the resin agitated for 6 h. The resin was separated by filtration and washed with AcOH/DCM (1:1, 3×) and MeCN/DCM (1:1, 3×). The solvents were evaporated under reduced pressure and the crude product was purified by means of HPLC. The purified product was lyophilized.

4. *Synthesis of Peptides*. *(*S*)-2-{[({[(*S*)-2-Amino-1-oxopropyl]amino}cyclopentyl)-carbonyl]amino}-3-phenylpropanoic Acid (H-Ala-Acp-Phe-OH;* **11a***)*. PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *5* and *6* to yield **11a** (21.0 mg, 37%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS (method B): t_R = 7.7 min, *m/z* = 348 (100, $[M + H]^+$), 183 (76, $[M – Phe]^+$), 155 (20, $[M – Phe – CO]^+$). IR (KBr): 3397*m*, 3262*s*, 3076*s*, 2962*m*, 2876*m*, 1731*s*, 1664*sh*, 1647v*s*, 1561*m*, 1517*s*, 1490*m*, 1455*m*, 1442*m*, 1382*w*, 1331*w*, 1266*m*, 1238*m*, 1202v*s*, 1141*s*, 1004*w*, 982*w*, 845*w*, 802*w*, 734*w*, 724*w*, 699*w*. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): 12.84 (br. *s*, COOH); 8.53, 8.48 (2*s*, NH(Acp)); 8.06 (br. *s*, NH_3(Ala)); 7.36 (*d*, *J* = 7.7, NH(Phe)); 7.27–7.17 (*m*, 5 arom. H); 4.44 (*ddd*, *J* = 7.7, 7.7, 5.6, CH(α)(Phe)); 3.82 (br. *s*, CH(α)(Ala)); 3.07 (*dd*, ^2J = 13.8, *J* = 5.6, 1 H of CH_2(Phe)); 2.94 (*dd*, ^2J = 13.8, *J* = 7.7, 1 H of CH_2(Phe)); 2.14–2.09 (*m*, 1 H of 4 CH_2(Acp)); 1.89–1.82 (*m*, 3 H of 4 CH_2(Acp)); 1.60–1.59 (*m*, 4 H of 4 CH_2(Acp)); 1.33 (*d*, *J* = 7.0, Me(Ala)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 172.7 (*s*, CO(Phe)); 172.2 (*s*, CO(Acp)); 169.3 (*s*, CO(Ala)); 137.4 (*s*, arom. C); 129.3, 128.1, 126.4 (3*d*, 5 arom. CH); 66.4 (*s*, C(α)(Acp)); 53.4 (*d*, CH(α)(Phe)); 48.3 (*d*, CH(α)(Ala)); 36.9 (*t*, CH_2(Phe)); 36.3, 35.3, 23.7 (3*t*, 4 CH_2(Acp)); 16.9 (*q*, Me(Ala)). ESI-MS: 370 (8, $[M + Na]^+$), 348 (100, $[M + H]^+$), 183 (13, $[M – Phe]^+$), 151 (24).

*(*S*)-2-{[(4-{[(*S*)-2-Amino-1-oxopropyl]amino}-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)carbon-yl]amino}-3-phenylpropanoic Acid (H-Ala-Thp-Phe-OH;* **11b***)*. PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *5* and *6* to yield **11b** (22.5 mg, 38%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS (method B): t_R = 6.1 min, *m/z* = 364 (100, $[M + H]^+$), 199 (58, $[M – Phe]^+$). IR (KBr): 3425*s*, 3243*s*, 3061v*s*, 3033v*s*, 2875*s*, 2616*w*, 1724v*s*, 1678v*s*, 1533v*s*, 1499*s*, 1455*m*, 1444*m*, 1429*m*, 1394*m*, 1356*m*, 1331*w*, 1302*m*, 1261*s*, 1244*s*, 1202v*s*, 1141*s*, 1102*s*, 1029*w*, 1019*w*, 969*w*, 843*w*, 800*w*, 723*m*, 701*m*. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): 12.79 (br. *s*, COOH); 8.43 (*s*,

NH(Thp)); 8.06 (br. *s*, NH_3(Ala)); 7.52 (*d*, *J* = 8.0, NH(Phe)); 7.28–7.51 (*m*, 5 arom. H); 4.46 (*ddd*, *J* = 8.3, 8.0, 5.4, CH(α)(Phe)); 3.95 (*q*, *J* = 6.7, CH(α)(Ala)); 3.68–3.65, 3.59–3.59, 3.52–3.46 (3*m*, 2 CH_2O(Thp)); 3.08 (*dd*, ^2J = 13.8, *J* = 5.4, 1 H of CH_2(Phe)); 2.95 (*dd*, ^2J = 13.8, *J* = 8.3, 1 H of CH_2(Phe)); 1.99–1.94, 1.87–1.76 (2*m*, 2 C*H*$_2$$CH_2O$(Thp)); 1.38 (*d*, *J* = 7.0, Me(Ala)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 172.6 (*s*, CO(Phe)); 171.9 (*s*, CO(Thp)); 169.5 (*s*, CO(Ala)); 137.4 (*s*, arom. C); 129.3, 128.1, 126.4 (3*d*, 5 arom. CH); 62.6, 62.3 (2*t*, 2 CH_2O(Thp)); 57.0 (*s*, C(α)(Thp)); 53.3 (*d*, CH(α)(Phe)); 48.4 (*d*, CH(α)(Ala)); 36.9 (*t*, CH_2(Phe)); 31.9, 31.3 (2*t*, 2 *C*$H_2$$CH_2O$(Thp)); 17.1 (*q*, Me(Ala)). ESI-MS: 364 (100, [*M* + H]$^+$).

*(*S*)-2-[((*S*)-2-{[(*S*)-2-Amino-1-oxopropyl]amino}-2-benzyl-1-oxopropyl)amino]-4-methylpentanoic Acid and (*S*)-2-[((*R*)-2-{[(*S*)-2-Amino-1-oxopropyl]amino}-2-benzyl-1-oxopropyl)amino]-4-methylpentanoic Acid (H-Ala-Phe(2Me)-Leu-OH; (*S*,*S*,*S*)-***13*** and (*S*,*R*,*S*)-***13*****).** PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *5* and *6* to yield (*S*,*S*,*S*)-**13** and (*S*,*R*,*S*)-**13** in a 1:1 ratio as colorless powders after prep. HPLC purification and lyophilization (29 mg, 49% altogether).

Data of (*S*,*S*,*S*)-**13**. HPLC-MS (method B): t_R = 8.8 min, *m*/*z* = 364 (100, [*M* + H]$^+$). IR (KBr): 3045*sh*, 3281*s*, 3066*s*, 3033*s*, 2960*s*, 2874*s*, 2623*w*, 1672v*s*, 1528v*s*, 1454*m*, 1388*m*, 1329*w*, 1268*m*, 1238*m*, 1202v*s*, 1141v*s*, 1031*w*, 1004*w*, 969*w*, 928*w*, 879*w*, 838*w*, 800*w*, 740*w*, 722*m*, 706*m*. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): *ca*. 9.5–8.0 (br. *s*, NH_3(Ala)); 8.09 (*s*, NH(Phe(2Me))); 7.82 (*d*, *J* = 8.2, NH(Leu)); 7.27–7.21, 7.12–7.11 (2*m*, 5 arom. H); 4.36–4.32 (*m*, CH(α)(Leu)); 3.81–3.80 (*m*, CH(α)(Ala)); 3.36, 3.18 (*AB*, *J* = 13.5, PhC*H*$_2$); 1.68–1.59 (*m*, CH(γ)(Leu), 1 H of CH_2(Leu)); 1.52–1.41 (*m*, 1 H of CH_2(Leu)); 1.31 (*s*, Me(Phe(2Me))); 1.30 (*d*, *J* = 7.0, Me(Ala)); 0.88, 0.86 (2*d*, *J* = 6.5, 2 Me(Leu)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 174.3 (*s*, CO(Leu)); 172.5 (*s*, CO(Phe(2Me))); 169.4 (*s*, CO(Ala)); 136.8 (*s*, arom. C); 130.6, 127.8, 126.4 (3*d*, 5 arom. CH); 59.6 (*s*, C(α)(Phe(2Me))); 50.5 (*d*, CH(α)(Leu)); 48.8 (*d*, CH(α)(Ala)); 40.1 (*t*, CH_2(Leu)); 38.8 (*t*, Ph*C*H_2); 24.0 (*d*, CH(γ)(Leu)); 23.7 (*q*, Me(Phe(2Me))); 23.0, 21.4 (2*q*, 2 Me(Leu)); 17.1 (*q*, Me(Ala)). ESI-MS: 408 (12, [*M* – H + 2 Na]$^+$), 386 (100, [*M* + Na]$^+$), 364 (11, [*M* + H]$^+$), 293 (7, [*M* – Ala + H]$^+$), 233 (8, [*M* – Leu]$^+$), 205 (23, [*M* – Leu – CO]$^+$), 134 (28).

Data of (*S*,*R*,*S*)-**13**. HPLC-MS (method B): t_R = 9.7 min, *m*/*z* = 364 (100, [*M* + H]$^+$). IR (KBr): 3291*s*, 3066*s*, 3034*s*, 2961*s*, 2875*s*, 2618*w*, 1720*s*, 1670v*s*, 1525v*s*, 1468*m*, 1454*m*, 1387*m*, 1329*w*, 1269*m*, 1202v*s*, 1143v*s*, 1031*w*, 1004*w*, 968*w*, 927*w*, 838*w*, 800*w*, 742*w*, 723*m*, 703*m*. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): 8.11 (*s*, NH_3(Ala)); 8.02 (*s*, NH(Phe(2Me))); 8.00 (*d*, *J* = 7.9, NH(Leu)); 7.25–7.19, 7.10–7.09 (2*m*, 5 arom. H); 4.29–4.25 (*m*,

CH(α)(Leu)); 3.93–3.91 (*m*, CH(α)(Ala)); 3.34 (*s*, PhC*H*$_2$); 1.71–1.65 (*m*, CH(γ)(Leu), 1 H of CH$_2$(Leu)); 1.55–1.52 (*m*, 1 H of CH$_2$(Leu)); 1.45 (*s*, Me(Phe(2Me))); 1.24 (*d*, *J* = 6.9, Me(Ala)); 0.92, 0.88 (2*d*, *J* = 6.4, 2 Me(Leu)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 173.9 (*s*, CO(Leu)); 172.5 (*s*, CO(Phe(2Me))); 168.8 (*s*, CO(Ala)); 136.7 (*s*, arom. C); 130.1, 127.8, 126.4 (3*d*, 5 arom. CH); 60.2 (*s*, C(α)(Phe(2Me))); 50.8 (*d*, CH(α)(Leu)); 48.4 (*d*, CH(α)(Ala)); 39.8 (*t*, CH$_2$(Leu)); 39.7 (*t*, PhCH$_2$); 24.3 (*d*, CH(γ)(Leu)); 23.0 (*q*, Me(Phe(2Me))); 23.0, 21.1 (2*q*, 2 Me(Leu)); 17.2 (*q*, Me(Ala)). ESI-MS: 408 (5, [*M* – H + 2 Na]$^+$), 386 (100, [*M* + Na]$^+$), 364 (96, [*M* + H]$^+$), 293 (23, [*M* – Ala + H]$^+$), 233 (24, [*M* – Leu]$^+$), 205 (43, [*M* – Leu – CO]$^+$), 134 (63).

*(*S*)-2-[({[(2-{[(4-{[(*S*)-2-Amino-3-methyl-1-oxobutyl]amino}-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)carbonyl]amino}-1-oxoethyl)amino]cyclopentyl}carbonyl)amino]propanoic Acid (H-Val-Thp-Gly-Acp-Ala-OH;* **15***)*. PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *5*, *2*, *3*, *4*, *5* and *6* to yield **15** (17.0 mg, 23%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS (method B): t_R = 4.9 min, *m*/*z* = 484 (100, [*M* + H]$^+$). IR (KBr): 3337*s*, 3056*s*, 2971*s*, 2880*m*, 2642*w*, 1720*sh*, 1670v*s*, 1534v*s*, 1455*m*, 1430*w*, 1400*w*, 1379*w*, 1333*w*, 1297*m*, 1240*m*, 1202v*s*, 1141*s*, 1103*m*, 1027*w*, 961*w*, 935*w*, 840*w*, 800*w*, 722*m*. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz) (2 conformers): *ca.* 13.0–12.0 (br. *s*, COOH); 8.82 (*s*, NH(Thp)); 8.57 (w) (*s*, NH(Acp)); 8.11 (*s*, NH$_3$(Val)); 8.04 (*s*, NH(Gly)); 7.73 (s) (*s*, NH(Acp)); 7.53 (w), 7.38 (s) (2*d*, *J* = 7.3, NH(Ala)); 4.26 (w), 4.14 (s) (2*dq*, *J* = 7.3, 7.3, CH(α)(Ala)); 3.77–3.71, 3.66–3.53, 3.47–3.43 (3*m*, CH(α)(Val), CH$_2$(Gly), 2 CH$_2$O(Thp)); 2.23–2.18, 2.14–2.07 (2*m*, CH(β)(Val), 1 H of 2 C*H*$_2$CH$_2$O(Thp), 1 H of 2 CH$_2$C*H*$_2$C(α)(Acp)); 1.97–1.76 (*m*, 3 H of 2 C*H*$_2$CH$_2$O(Thp), 3 H of 2 CH$_2$C*H*$_2$C(α)(Acp)); 1.69–1.56 (*m*, 2 C*H*$_2$CH$_2$C(α)(Acp)); 1.27 (s), 1.24 (w) (2*d*, *J* = 7.3, Me(Ala)); 0.99 (s), 0.95 (s), 0.94 (w, w) (3*d*, *J* = 6.9, Me(Val)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz) (2 conformers): 173.9 (*s*, CO(Ala)); 173.3 (*s*, CO(Thp)); 173.2 (*s*, CO(Acp)); 168.7 (*s*, CO(Gly)); 168.5 (*s*, CO(Val)); 66.4 (w), 65.9 (s) (2*s*, C(α)(Acp)); 62.5, 62.4 (2*t*, 2 CH$_2$O(Thp)); 57.6 (s), 57.6 (w) (2*d*, CH(α)(Val)); 56.9 (*s*, C(α)(Thp)); 47.7 (s), 47.6 (w) (2*d*, CH(α)(Ala)); 43.2 (*t*, CH$_2$(Gly)); 37.1, 35.2 (2*t*, 2 CH$_2$*C*H$_2$C(α)(Acp)); 32.8, 30.1 (2*t*, 2 *C*H$_2$CH$_2$O(Thp)); 29.6 (s), 29.5 (w) (2*d*, CH(β)(Val)); 24.1 (s), 24.0 (s), 23.9 (w), 23.8 (w) (4*t*, 2 *C*H$_2$CH$_2$C(α)(Acp)); 18.6 (s), 18.2 (w), 17.5 (w), 17.2 (s) (4*q*, 2 Me(Val)); 17.1 (w), 17.0 (s) (2*q*, Me(Ala)). ESI-MS: 484 (100, [*M* + H]$^+$), 300 (20).

*(*S*)-2-[({4-[((*S*)-2-{[(4-{[(*S*)-2-Amino-1-oxopropyl]amino}-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)carbonyl]amino}-3-methyl-1-oxobutyl)amino]-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl)amino]-3-phenylpropanoic Acid (H-Ala-Thp-Val-Thp-Phe-OH;* **16***)*. PAM resin (200 mg,

0.124 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *5*, *2*, *3*, *4*, *5* and *6* to yield **16** (14.3 mg, 16%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS (method B): t_R = 8.3 min, *m/z* = 590 (100, $[M + H]^+$), 425 (98, $[M - Phe]^+$), 298 (48, $[M - (Thp–Phe)]^+$). IR (KBr): 3426*s*, 3306*s*, 3061*s*, 2967*s*, 2875*m*, 2620*w*, 1720*s*, 1671v*s*, 1533v*s*, 1469*m*, 1444*m*, 1429*m*, 1393*m*, 1356*w*, 1302*m*, 1259*m*, 1245*m*, 1203v*s*, 1189*sh*, 1142*s*, 1104*s*, 1029*w*, 968*w*, 946*w*, 917*w*, 838*w*, 800*w*, 722*m*, 702*w*. ^{1}H-NMR ((D_6)DMSO, 600 MHz): *ca*. 13.0–12.5 (br. *s*, COOH); 8.52 (*s*, NH(Thp^1)); 8.07 (*s*, NH_3(Ala)); 8.03 (*s*, NH(Thp^2)); 7.45 (*d*, *J* = 7.9, NH(Phe)); 7.38 (*d*, *J* = 7.7, NH(Val)); 7.27–7.16 (*m*, 5 arom. H)); 4.43 (*ddd*, *J* = 7.9, 7.9, 5.9, CH(α)(Phe)); 4.17 (*dd*, *J* = 7.3, 7.3, CH(α)(Val)); 3.99–3.97 (*m*, CH(α)(Ala)); 3.71–3.41 (*m*, 4 CH_2O(Thp)); 3.02 (*dd*, ^2J = 13.8, *J* = 5.9, 1 H of CH_2(Phe)); 2.95 (*dd*, ^2J = 13.8, *J* = 7.5, 1 H of CH_2(Phe)); 2.07–2.04 (*m*, CH(β)(Val)); 2.01–1.84 (*m*, 4 C*H*$_2$CH$_2$O(Thp)); 1.39 (*d*, *J* = 7.0, Me(Ala)); 0.87, 0.82 (2*d*, *J* = 6.7, 2 Me(Val)). ^{13}C-NMR ((D_6)DMSO, 150 MHz): 172.5 (*s*, 2 CO(Thp)); 172.4 (*s*, CO(Phe)); 171.1 (*s*, CO(Val)); 169.7 (*s*, CO(Ala)); 137.2 (*s*, arom. C); 129.1, 128.1, 126.3 (3*d*, 5 arom. CH); 62.4, 62.3, 62.3 (3*t*, 4 CH_2O(Thp)); 58.4 (*d*, CH(α)(Val)); 57.1, 56.9 (2*s*, 2 C(α)(Thp)); 53.3 (*d*, CH(α)(Phe)); 48.5 (*d*, CH(α)(Ala)); 36.9 (*t*, CH_2(Phe)); 31.9, 31.7, 31.3, 31.1 (4*t*, 4 *C*H$_2$CH$_2$O(Thp)); 30.0 (*d*, CH(β)(Val)); 19.5, 18.2 (2*q*, 2 Me(Val)); 17.1 (*q*, Me(Ala)). ESI-MS: 590 (100, $[M + H]^+$), 425 (27, $[M - Phe]^+$).

*(S)-2-{[(4-{[(S)-2-({[({(S)-2-[(2-{[(S)-2-Amino-1-oxopropyl]amino}-2-methyl-1-oxopropyl)amino]-3-methyl-1-oxobutyl}amino)cyclopentyl]carbonyl}amino)-1-oxoethyl]amino}-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)carbonyl]amino}-4-methylpentanoic Acid (H-Ala-Aib-Val-Acp-Gly-Thp-Leu-OH;* **17***).* PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *5*, *2*, *3*, *4*, *5*, *2*, *3*, *4*, *5* and *6* to yield **17** (20.2 mg, 21%) as a colorless powder after prep. HPLC purification and lyophilization. For the 'conventional' coupling of Gly and Leu, the coupling was performed two times (1 × 2 h and 1 × overnight). HPLC-MS (method B): t_R = 11.9 min, *m/z* = 682 (100, $[M + H]^+$). IR (KBr): 3314*s*, 3054*m*, 2962*s*, 2874*m*, 1722*sh*, 1662v*s*, 1536v*s*, 1470*m*, 1448*m*, 1389*m*, 1368*w*, 1329*w*, 1295*m*, 1267*m*, 1250*sh*, 1202*s*, 1172*m*, 1141*m*, 1107*w*, 1062*w*, 1029*w*, 977*w*, 947*w*, 929*w*, 851*w*, 835*w*, 800*w*, 722*w*. ^{1}H-NMR ((D_6)DMSO, 600 MHz): *ca*. 12.6–12.1 (br. *s*, COOH); 8.69 (*s*, NH(Aib)); 8.49 (*s*, NH(Acp)); 8.23 (br. *s*, NH_3(Ala)); 8.10 (*t*, *J* = 5.6, NH(Gly)); 7.90 (*d*, *J* = 6.1, NH(Val)); 7.47 (*s*, NH(Thp)); 7.42 (*d*, *J* = 8.1, NH(Leu)); 4.23–4.19 (*m*, CH(α)(Leu)); 3.90–3.88 (*m*, CH(α)(Ala)); 3.79 (*m*, CH(α)(Val)); 3.68–3.64 (*m*, 3 H of 2 CH_2O(Thp)); 3.60 (*d*, *J* = 5.6, CH_2(Gly)); 3.57–3.45 (*m*, 1 H of 2 CH_2O(Thp)); 2.30–2.28 (*m*, 1 H of 2 CH_2CH_2C(α)(Acp)); 2.19–2.16 (*m*, CH(β)(Val)); 2.09–2.02 (*m*, 2 H of 2 C*H*$_2$CH$_2$O(Thp)); 2.00–1.88 (*m*, 1 H of 2

C*H*$_2$CH$_2$O(Thp), 3 H of 2 CH$_2$C*H*$_2$C(α)(Acp)); 1.84–1.80 (*m*, 1 H of 2 C*H*$_2$CH$_2$O(Thp)); 1.72–1.60 (*m*, CH(γ)(Leu), 1 H of CH$_2$(Leu), 4 H of 2 C*H*$_2$CH$_2$C(α)(Acp)); 1.49–1.46 (*m*, 1 H of CH$_2$(Leu)); 1.44, 1.40 (2*s*, 2 Me(Aib)); 1.37 (*d*, *J* = 6.9, Me(Ala)); 0.88, 0.87 (2*d*, *J* = 6.7, 2 Me(Val)); 0.86, 0.82 (2*d*, *J* = 6.4, 2 Me(Leu)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 175.0 (*s*, CO(Acp)); 174.7 (*s*, CO(Aib)); 173.8 (*s*, CO(Leu)); 173.1 (*s*, CO(Thp)); 172.4 (*s*, CO(Val)); 169.5 (*s*, CO(Ala)); 168.9 (*s*, CO(Gly)); 66.1 (*s*, C(α)(Acp)); 62.4, 62.3 (2*t*, 2 CH$_2$O(Thp)); 60.1 (*d*, CH(α)(Val)); 57.1 (*s*, C(α)(Thp)); 56.4 (*s*, C(α)(Aib)); 50.3 (*d*, CH(α)(Leu)); 48.5 (*d*, CH(α)(Ala)); 44.1 (*t*, CH$_2$(Gly)); 39.8 (*t*, CH$_2$(Leu)); 36.9, 35.7 (2*t*, 2 CH$_2$*C*H$_2$C(α)(Acp)); 32.7, 30.3 (2*t*, 2 *C*H$_2$CH$_2$O(Thp)); 28.3 (*d*, CH(β)(Val)); 25.4, 24.5 (2*q*, 2 Me(Aib)); 24.1, 24.1, 24.0 (*d*, 2*t*, CH(γ)(Leu), 2 *C*H$_2$CH$_2$C(α)(Acp)); 23.0, 21.3 (2*q*, 2 Me(Leu)); 19.4, 19.3 (2*q*, 2 Me(Val)); 16.7 (*q*, Me(Ala)). ESI-MS: 682 (100, [*M* + H]$^+$).

*(*S*)-2-{[(4-{[(*S*)-2-({[({(*S*)-2-[(2-{[(*S*)-2-Amino-1-oxopropyl]amino}-2-methyl-1-oxopropyl)amino]-3-methyl-1-oxobutyl}amino)cyclopentyl]carbonyl}amino)-3-phenyl-1-oxopropyl]amino}-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)carbonyl]amino}-4-methylpentanoic Acid (H-Ala-Aib-Val-Acp-Phe-Thp-Leu-OH;* **18***)*. PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *5*, *2*, *3*, *4*, *5*, *2*, *3*, *4*, *5* and *6* to yield **18** (14.2 mg, 13%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS (method B): t_R = 11.4 min, *m*/*z* = 772 (100, [*M* + H]$^+$). IR (KBr): 3431*s*, 3319*s*, 3062*m*, 3032*m*, 2962*s*, 2874*m*, 1726*sh*, 1662v*s*, 1532v*s*, 1469*m*, 1454*m*, 1445*m*, 1389*w*, 1367*w*, 1326*w*, 1293*sh*, 1266*m*, 1244*m*, 1202*s*, 1140*s*, 1108*w*, 1029*w*, 978*w*, 927*w*, 838*w*, 800*w*, 722*w*, 700*w*. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): *ca.* 12.8–12.2 (br. *s*, COOH); 8.78 (br. *s*, NH(Aib)); 8.15 (br. *s*, NH$_3$(Ala)); 8.04 (br. *s*, NH(Val), NH(Acp)); 7.72 (br. *s*, NH(Phe)); 7.61 (*s*, NH(Thp)); 7.26–7.24 (*m*, 2 arom. CH); 7.21–7.17 (*m*, NH(Leu), 3 arom. CH); 4.34–4.30 (*m*, CH(α)(Leu)); 4.23 (br. *s*, CH(α)(Phe)); 3.87 (br. *s*, CH(α)(Ala)); 3.83–3.81 (*m*, CH(α)(Val)); 3.68–3.65 (*m*, 3 H of 2 CH$_2$O(Thp)); 3.34–3.31 (*m*, 1 H of CH$_2$(Phe)); 3.26–3.22 (*m*, 1 H of 2 CH$_2$O(Thp)); 2.92–2.87 (*m*, 1 H of CH$_2$(Phe)); 2.16–2.15 (*m*, 2 H of 2 C*H*$_2$CH$_2$O(Thp)); 2.08–2.04 (*m*, CH(β)(Val), 1 H of 4 CH$_2$(Acp)); 1.99–1.94 (*m*, 1 H of 2 C*H*$_2$CH$_2$O(Thp), 1 H of 4 CH$_2$(Acp)); 1.79–1.71 (*m*, CH(γ)(Leu), 1 H of 2 C*H*$_2$CH$_2$O(Thp), 2 H of 4 CH$_2$(Acp)); 1.69–1.58 (*m*, 1 H of CH$_2$(Leu), 4 H of 4 CH$_2$(Acp)); 1.49–1.44 (*m*, 1 H of CH$_2$(Leu)); 1.49 (*s*, 1 Me of 2 Me(Aib)); 1.37 (*d*, *J* = 6.9, Me(Ala)); 1.36 (*s*, 1 Me of 2 Me(Aib)); 0.91 (*d*, *J* = 6.8, 1 Me of 2 Me(Val)); 0.88 (*d*, *J* = 6.7, 1 Me of 2 Me(Leu)); 0.85 (*d*, *J* = 6.7, 1 Me of 2 Me(Val), 1 Me of 2 Me(Leu)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 175.9 (*s*, CO(Aib)); 174.2 (*s*, CO(Acp)); 173.9 (*s*, CO(Leu)); 172.9 (*s*, CO(Thp)); 172.6 (*s*, CO(Val)); 170.3 (*s*, CO(Phe)); 169.4 (*s*, CO(Ala)); 138.4 (*s*, arom. C); 129.0, 128.2, 126.1 (3*d*, 5 arom. CH); 66.3

(*s*, C(α)(Acp)); 62.5, 62.3 (2*t*, 2 CH_2O(Thp)); 60.8 (*d*, CH(α)(Val)); 57.1 (*s*, C(α)(Thp)); 56.2 (*s*, C(α)(Aib)); 54.9 (*d*, CH(α)(Phe)); 49.8 (*d*, CH(α)(Leu)); 48.4 (*d*, CH(α)(Ala)); 40.2 (*t*, CH_2(Leu)); 36.1, 36.0 (2*t*, 2 CH_2CH_2C(α)(Acp)); 35.5 (*t*, CH_2(Phe)); 33.7, 28.7 (2*t*, 2 CH_2CH_2O(Thp)); 28.5 (*d*, CH(β)(Val)); 25.3, 24.3 (2*q*, 2 Me(Aib)); 24.1, 24.0 (2*t*, 2 CH_2CH_2C(α)(Acp)); 23.7 (*d*, CH(γ)(Leu)); 23.1, 21.1 (2*q*, 2 Me(Leu)); 19.3, 18.4 (2*q*, 2 Me(Val)); 16.7 (*q*, Me(Ala)). ESI-MS: 772 (100, $[M + H]^+$).

2-({2-[(2-Amino-2-methyl-1-oxopropyl)amino]-2-methyl-1-oxopropyl}amino)-2-methylpropanoic Acid (H-Aib-Aib-Aib-OH; **19***).* PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *3* and *6* to yield **19** (15.9 mg, 33%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS (method A): t_R = 1.3 min, *m*/*z* = 274 (100, $[M + H]^+$). IR (KBr): 3508*m*, 3359*s*, 3262*m*, 3119*s*, 3064*s*, 2994*s*, 2947*s*, 2604*w*, 1719v*s*, 1667v*s*, 1519v*s*, 1472*m*, 1440*m*, 1406*w*, 1390*w*, 1367*w*, 1258*s*, 1203v*s*, 1179v*s*, 1144v*s*, 947*w*, 925*w*, 911*w*, 839*w*, 801*w*, 773*w*, 723*m*. ^{1}H-NMR ((D_6)DMSO, 300 MHz): *ca*. 9.0–7.0 (br. *s*, NH_3); 8.00, 7.38 (2*s*, 2 NH); 1.49, 1.41, 1.38 (3*s*, 6 Me). ^{13}C-NMR ((D_6)DMSO, 75 MHz): 175.8, 172.6, 170.7 (3*s*, 3 CO); 56.6, 56.5, 55.2 (3*s*, 3 C(α)); 24.5, 24.4, 23.3 (3*q*, 6 Me). ESI-MS: 274 (100, $[M + H]^+$), 170 (4, $[M – Aib]^+$).

2-[(2-{[(S)-2-Amino-1-oxopropyl]amino}-2-methyl-1-oxopropyl)amino]-2-methylpropanoic Acid (H-Ala-Aib-Aib-OH; **20***).* PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *3* and *6* to yield **20** (19.0 mg, 41%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS (method A): t_R = 1.3 min, *m*/*z* = 260 (100, $[M + H]^+$). IR (KBr): 3284*s*, 3072*s*, 2993*s*, 2945*s*, 2631*w*, 1726v*s*, 1673v*s*, 1530v*s*, 1471*m*, 1458*m*, 1441*m*, 1389*m*, 1368*m*, 1264*s*, 1204v*s*, 1141v*s*, 1005*w*, 930*w*, 880*w*, 838*m*, 801*m*, 768*w*, 723*m*. ^{1}H-NMR ((D_6)DMSO, 600 MHz): *ca*. 8.8–7.5 (br. *s*, NH_3(Ala)); 8.38 (*s*, NH(Aib^2)); 7.46 (*s*, NH(Aib^3)); 3.85 (*q*, *J* = 6.9, CH(α)(Ala)); 1.41–1.34 (*m*, 4 Me(Aib), Me(Ala)). ^{13}C-NMR ((D_6)DMSO, 150 MHz): 175.7 (*s*, CO(Aib^3)); 172.5 (*s*, CO(Aib^2)); 168.9 (*s*, CO(Ala)); 56.3 (*s*, C(α)(Aib^2)); 55.2 (*s*, C(α)(Aib^3)); 48.3 (*d*, CH(α)(Ala)); 24.8, 24.5, 24.5, 24.4 (4*q*, 4 Me(Aib)); 17.1 (*q*, Me(Ala)). ESI-MS: 519 (36, $[2M + H]^+$), 260 (100, $[M + H]^+$).

2-({2-[(2-{[(S)-2-Amino-1-oxopropyl]amino}-2-methyl-1-oxopropyl)amino]-2-methyl-1-oxopropyl}amino)-2-methylpropanoic Acid (H-Ala-Aib-Aib-Aib-OH; **21***).* PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *3*, *4*, *3* and *6* to yield **21** (7 mg, 12%) as a colorless powder after prep. HPLC purification and lyophilization. Additional **20** was isolated as a colorless powder (16 mg, 35%). HPLC-MS (method A): t_R = 1.5 min, *m*/*z* = 345 (100, $[M + H]^+$). IR(KBr): 3432v*s*, 3262v*s*, 3120v*s*, 2992v*s*, 2939*s*, 1670v*s*, 1543v*s*, 1535v*s*, 1469*s*, 1460*s*, 1399v*s*, 1367*m*, 1261*m*, 1204v*s*, 1182*s*, 1141*s*, 1089*w*, 1048*w*, 1025*w*,

1005*w*, 722*w*. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): *ca.* 12.2–11.8 (br. *s*, COOH); 8.61 (*s*, NH(Aib)); 8.04 (br. *s*, NH$_3$(Ala)); 7.44, 7.31 (2*s*, 2 NH(Aib)); 3.87 (br. *s*, CH(α)(Ala)); 1.39–1.34 (*m*, 6 Me(Aib), Me(Ala)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 174.4, 172.2, 171.1, 168.1 (4*s*, 4 CO); 55.2, 54.6, 53.8 (3*s*, 3 C(α)(Aib)); 47.1 (*d*, CH(α)(Ala)); 23.9, 23.6, 23.5, 23.3, 23.3, 23.2 (6*q*, 6 Me(Aib)); 15.8 (*q*, Me(Ala)). ESI-MS: 345 (100, [*M* + H]$^+$).

2-{[2-({2-[((S)-2-{[(S)-2-Amino-1-oxopropyl]amino}-3-methyl-1-oxobutyl)amino]-2-methyl-1-oxopropyl}amino)-2-methyl-1-oxopropyl]amino}-2-methylpropanoic Acid (H-Ala-Val-Aib-Aib-Aib-OH; ***22****)*. PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *5*, *2*, *3*, *4*, *3*, *4*, *3* and *6* to yield **22** (11.0 mg, 16%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS (method A): t_R = 8.4 min, *m*/*z* = 444 (100, [*M* + H]$^+$). IR (KBr): 3431*s*, 3304*s*, 3063*s*, 2987*s*, 2942*s*, 2883*m*, 2629*w*, 1720*sh*, 1667v*s*, 1534v*s*, 1469*m*, 1389*m*, 1366*m*, 1203v*s*, 1181v*s*, 1140*s*, 1010*w*, 935*w*, 837*w*, 800*w*, 776*w*, 722*m*. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): *ca.* 9.5–7.5 (br. *s*, NH$_3$(Ala)); 8.40 (*s*, NH(Aib^3)); 8.33 (*d*, *J* = 7.6, NH(Val)); 7.34 (*s*, NH(Aib^5)); 7.13 (*s*, NH(Aib^4)); 4.11 (*dd*, *J* = 7.2, 7.2, CH(α)(Val)); 3.95 (*q*, *J* = 6.9, CH(α)(Ala)); 2.05 (*dsept.*, *J* = 6.8, 6.8, CH(β)(Val)); 1.33–1.29 (*m*, 6 Me(Aib), Me(Ala)); 0.93, 0.91 (2*d*, *J* = 7.0, 2 Me(Val)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 175.6 (*s*, CO(Aib^5)); 173.3 (*s*, CO(Aib^4)); 172.6 (*s*, CO(Aib^3)); 170.9 (*s*, CO(Val)); 169.8 (*s*, CO(Ala)); 58.3 (*d*, CH(α)(Val)); 56.1 (*s*, C(α)(Aib^3)); 55.7 (*s*, C(α)(Aib^4)); 54.8 (*s*, C(α)(Aib^5)); 47.9 (*d*, CH(α)(Ala)); 30.0 (*d*, CH(β)(Val)); 25.0 (*q*, 1 Me of 2 Me(Aib^3)); 24.9 (*q*, 1 Me of 2 Me(Aib^4)); 24.8, 24.5 (2*q*, 2 Me(Aib^5)); 24.4 (*q*, 1 Me of 2 Me(Aib^4)); 24.2 (*q*, 1 Me of 2 Me(Aib^3)); 19.2, 18.2 (2*q*, 2 Me(Val)); 17.4 (*q*, Me(Ala)). ESI-MS: 444 (100, [*M* + H]$^+$).

(S)-2-[(2-{[2-({(S)-2-[((S)-2-{[(S)-2-Amino-1-oxopropyl]amino}-3-methyl-1-oxobutyl)-amino]-3-phenyl-1-oxopropyl}amino)-2-methyl-1-oxopropyl]amino}-2-methyl-1-oxopropyl)-amino]-4-methylpentanoic Acid (H-Ala-Val-Phe-Aib-Aib-Leu-OH; ***23****)*. PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *5*, *2*, *5*, *2*, *3*, *4*, *3*, *4*, *5* and *6* to yield **23** (5.3 mg, 6%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS (method B): t_R = 11.1 min, *m*/*z* = 619 (100, [*M* + H]$^+$). IR (KBr): 3421*s*, 3312*s*, 3065*m*, 3034*m*, 2964*s*, 2939*m*, 2876*m*, 1668v*s*, 1534v*s*, 1468*m*, 1460*m*, 1442*m*, 1388*m*, 1367*w*, 1203v*s*, 1190*sh*, 1140*s*, 837*w*, 800*w*, 745*w*, 722*w*, 700*w*. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): *ca.* 12.4–12.1 (br. *s*, COOH); 8.32 (*d*, *J* = 5.7, NH(Phe)); 8.26 (*d*, *J* = 8.6, NH(Val)); 8.19 (*s*, NH(Aib^4)); 8.04 (br. *s*, NH$_3$(Ala)); 7.32 (*s*, NH(Aib^5)); 7.30–7.18 (*m*, 5 arom. H, NH(Leu)); 4.41 (*ddd*, *J* = 8.1, 6.3, 6.3, CH(α)(Phe)); 4.15 (*dd*, *J* = 8.2, 8.2, CH(α)(Val)); 4.13–4.10 (*m*, CH(α)(Leu)); 3.93–3.90 (*m*, CH(α)(Ala)); 2.95 (*dd*, *J* = 13.9, 6.5, 1 H of CH$_2$(Phe)); 2.88 (*dd*,

J = 13.9, 8.5, 1 H of CH_2(Phe)); 1.92 (*dsept.*, *J* = 7.0, 7.0, CH(β)(Val)); 1.72–1.63 (*m*, CH(γ)(Leu), 1 H of CH_2(Leu)); 1.47–1.42 (*m*, 1 H of CH_2(Leu)); 1.34, 1.31 (2*s*, 2 Me(Aib^5)); 1.26 (*d*, *J* = 7.0, Me(Ala)); 1.19, 1.12 (2*s*, 2 Me(Aib^4)); 0.90 (*d*, *J* = 6.7, 1 Me of 2 Me(Val)); 0.87 (*d*, *J* = 6.5, 1 Me of 2 Me(Leu)); 0.84 (*d*, *J* = 6.7, 1 Me of 2 Me(Val)); 0.81 (*d*, *J* = 6.5, 1 Me of 2 Me(Leu)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 174.1 (*s*, CO(Aib^5)); 173.8 (*s*, CO(Leu)); 172.6 (*s*, CO(Aib^4)); 171.0 (*s*, CO(Phe)); 170.7 (*s*, CO(Val)); 169.2 (*s*, CO(Ala)); 137.0 (*s*, arom. C); 129.1, 127.9, 126.2 (3*d*, 5 arom. CH); 57.8 (*d*, CH(α)(Val)); 55.9 (*s*, C(α)(Aib^4)); 55.6 (*s*, C(α)(Aib^5)); 54.5 (*d*, CH(α)(Phe)); 50.3 (*d*, CH(α)(Leu)); 47.8 (*d*, CH(α)(Ala)); 39.6 (*t*, CH_2(Leu)); 36.9 (*t*, CH_2(Phe)); 30.3 (*d*, CH(β)(Val)); 25.9 (*q*, 1 Me of 2 Me(Aib^5)); 25.8 (*q*, 1 Me of 2 Me(Aib^4)); 23.8 (*d*, CH(γ)(Leu)); 23.5 (*q*, 1 Me of 2 Me(Aib^5)); 23.4 (*q*, 1 Me of 2 Me(Aib^4)); 23.0, 21.0 (2*q*, 2 Me(Leu)); 19.2, 18.5 (2*q*, 2 Me(Val)); 17.3 (*q*, Me(Ala)). ESI-MS: 619 (100, $[M + H]^+$), 488 (21, $[M - \text{Leu}]^+$), 403 (10, $[M - (\text{Aib–Leu})]^+$).

*(*S*)-2-({2-[(2-{[2-({(*S*)-2-[((*S*)-2-{[(*S*)-2-Amino-1-oxopropyl]amino}-3-methyl-1-oxobutyl)amino]-3-phenyl-1-oxopropyl}amino)-2-methyl-1-oxopropyl]amino}-2-methyl-1-oxopropyl)amino]-2-methyl-1-oxomethyl}amino)-4-methylpentanoic Acid (H-Ala-Val-Phe-Aib-Aib-Aib-Leu-OH;* **24***)*. PAM resin (200 mg, 0.124 mmol) was treated as described in *GP 1*, *2*, *5*, *2*, *5*, *2*, *3*, *4*, *3*, *4*, *3*, *4*, *5* and *6* to yield **24** (8.6 mg, 9%) as a colorless powder after prep. HPLC purification and lyophilization. HPLC-MS (method B): t_R = 11.1 min, *m*/*z* = 704 (100, $[M + H]^+$). IR (KBr): 3422*s*, 3307*s*, 3065*s*, 3033*s*, 2964*s*, 2941*s*, 2875*m*, 1667v*s*, 1532v*s*, 1468*m*, 1458*m*, 1442*m*, 1387*m*, 1366*m*, 1276*m*, 1203v*s*, 1188*s*, 1140*s*, 945*w*, 923*w*, 837*w*, 800*w*, 722*w*, 700*w*. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): *ca*. 12.3–12.1 (br. *s*, COOH); 8.44 (*s*, NH(Aib^4)); 8.42 (*d*, *J* = 4.9, NH(Phe)); 8.26 (*d*, *J* = 8.6, NH(Val)); 8.04 (br. *s*, NH_3(Ala)); 7.53, 7.41 (2*s*, NH(Aib^5), NH(Aib^6)); 7.40 (*d*, *J* = 7.9, NH(Leu)); 7.28–7.20 (*m*, 5 arom. H); 4.41 (*td*, *J* = 7.6, 5.1, CH(α)(Phe)); 4.20 (*dd*, *J* =8.1, 8.1, CH(α)(Val)); 4.18–4.14 (*m*, CH(α)(Leu)); 3.91 (br. *s*, CH(α)(Ala)); 2.96–2.89 (*m*, CH_2(Phe)); 1.93 (*dsept.*, *J* = 7.0, 7.0, CH(β)(Val)); 1.78–1.75 (*m*, CH(γ)(Leu)); 1.73–1.69, 1.44–1.39 (2*m*, CH_2(Leu)); 1.34, 1.33, 1.30, 1.26 (4*s*, 4 Me of 6 Me(Aib)); 1.26 (*d*, *J* = 6.5, Me(Ala)); 1.20, 1.14 (2*s*, 2 Me of 6 Me(Aib)); 0.94, 0.88 (2*d*, *J* = 6.8, 2 Me(Val)); 0.83, 0.81 (2*d*, *J* = 6.6, 2 Me(Leu)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 174.3, 174.1 (2*s*, 2 CO of 3 CO(Aib)); 174.0 (*s*, CO(Leu)); 173.0 (*s*, 1 CO of 3 CO(Aib)); 171.7 (*s*, CO(Phe)); 170.8 (*s*, CO(Val)); 169.2 (*s*, CO(Ala)); 136.8 (*s*, arom. C); 129.2, 127.9, 126.3 (3*d*, 5 arom. CH); 57.6 (*d*, CH(α)(Val)); 56.1, 55.8, 55.7 (3*s*, 3 C(α)(Aib)); 54.5 (*d*, CH(α)(Phe)); 50.2 (*d*, CH(α)(Leu)); 47.8 (*d*, CH(α)(Ala)); 39.8 (*t*, CH_2(Leu)); 36.7 (*t*, CH_2(Phe)); 30.3 (*d*, CH(β)(Val)); 26.7, 26.1, 25.9 (3*q*, 3 Me of 6 Me(Aib)); 23.6 (*d*, CH(γ)(Leu)); 23.4, 23.2 (2*q*, 2 Me of 6 Me(Aib)); 23.0 (*q*, 1 Me of 2

Me(Leu)); 22.9 (*q*, 1 Me of 6 Me(Aib)); 21.0 (*q*, 1 Me of 2 Me(Leu)); 19.2, 18.5 (2*q*, 2 Me(Val)); 17.3 (*q*, Me(Ala)). ESI-MS: 704 (100, $[M + H]^+$), 573 (21, $[M - Leu]^+$).

5. Derivatization of (*S*,*S*,*S*)-**13** *and* (*S*,*R*,*S*)-**13**. *(*S*)-2-{[(*S*)-2-({(*S*)-2-[4-(Bromobenzoyl)amino]-1-oxopropyl}amino)-2-benzyl-1-oxopropyl]amino}-4-methylpentanoic Acid (*p-*BrBz-Ala-Phe(2Me)-Leu-OH;* (*S*,*S*,*S*)-**14**) *and (*S*)-2-{[(*R*)-2-({(*S*)-2-[4-(Bromobenzoyl)amino]-1-oxopropyl}amino)-2-benzyl-1-oxopropyl]amino}-4-methylpentanoic Acid (*p-*BrBz-Ala-Phe(2Me)-Leu-OH;* (*S*,*R*,*S*)-**14**). At 0° 4-bromobenzoylchloride (8 mg, 0.036 mmol) was added to a mixture of (*S*,*S*,*S*)-**13** (*ca.* 12 mg, 0.025 mmol) and K_2CO_3 (12 mg, 0.087 mmol) and (*S*,*R*,*S*)-**13** (*ca.* 12 mg, 0.025 mmol) and K_2CO_3 (12 mg, 0.087 mmol), resp., in acetone (5 ml) and H_2O (1 ml), then stirred at r.t. for 2 h. The org. solvent was removed under reduced pressure and the residue acidified with diluted aq. HCl. The resulting precipitation was filtered and purified by prep. TLC (CH_2Cl_2/MeOH 10:1; 2 × dev.) yielding colorless powders (8 mg (59%) and 6 mg (44%), resp.). Suitable crystals for the X-ray crystal-structure determination of (*S*,*S*,*S*)-**14** were grown from MeOH/$CHCl_3$/Et_2O. *Data of (*S,S,S*)-***14***.* ESI-MS: 593 (10), 592 (30, $[M(^{81}Br) - H + 2\ Na]^+$), 591 (10), 590 (33, $[M(^{79}Br) - H + 2\ Na]^+$), 571 (42), 570 (100, $[M(^{81}Br) + Na]^+$), 569 (34), 568 (86, $[M(^{79}Br) + Na]^+$).

*Data of (*S,R,S*)-***14***.* ESI-MS: 593 (5), 592 (11, $[M(^{81}Br) - H + 2\ Na]^+$), 591 (5), 590 (11, $[M(^{79}Br) - H + 2\ Na]^+$), 571 (29), 570 (100, $[M(^{81}Br) + Na]^+$), 569 (26), 568 (72, $[M(^{79}Br) + Na]^+$).

6. X-Ray Crystal-Structure Determination of (*S*,*S*,*S*)-**14**, *(see Table 3 and Fig. 1)*[2]). A crystal of $C_{26}H_{32}BrN_3O_5 \cdot MeOH$, obtained from MeOH/$CHCl_3$/$Et_2O$, was used for a low-temperature X-ray structure determination. All measurements were made on a *Nonius KappaCCD* area-detector diffractometer [27] using graphite-monochromated MoK_α radiation (λ 0.71073 Å) and an *Oxford Cryosystems Cryostream 700* cooler. The data collection and refinement parameters are given in *Table 3* and a view of the molecule is shown in the *Fig.*

Data reduction was performed with *HKL Denzo* and *Scalepack* [28]. The intensities were corrected for *Lorentz* and polarization effects, and an absorption correction based on the multi-scan method [29] was applied. Equivalent reflections, other than *Friedel* pairs, were merged.

[2]) CCDC-287054 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the *Cambridge Crystallographic Data Center via* http://www.ccdc.cam.ac.uk/data_request/cif.

The structure was solved by direct methods using *SHELXS97* [30], which revealed the positions of all non-H-atoms. There are two symmetry-independent peptide and two MeOH molecules in the asymmetric unit. The atomic coordinates were tested carefully for a relationship from a higher symmetry space group by using the program *PLATON* [31], but none could be found. The non-hydrogen atoms were refined anisotropically. The hydroxy H-atoms of the peptide and MeOH molecules were placed in the positions indicated by a difference electron density map and their positions were allowed to refine together with individual isotropic displacement parameters. All remaining H-atoms were placed in geometrically calculated positions and refined using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to $1.2U_{eq}$ of its parent C-atom ($1.5U_{eq}$ for the methyl groups). The refinement of the structure was carried out on F^2 using full-matrix least-squares procedures, which minimised the function $\Sigma w(F_o^2 - F_c^2)^2$. A correction for secondary extinction was not applied. Eleven reflections, whose intensities were considered to be extreme outliers, were omitted from the final refinement. Refinement of the absolute structure parameter [32] yielded a value of 0.00(1), which confidently confirms that the refined model represents the true enantiomorph. Neutral atom scattering factors for non-H-atoms were taken from [33a], and the scattering factors for H-atoms were taken from [34]. Anomalous dispersion effects were included in F_c [35]; the values for f' and f'' were those of [33b]. The values of the mass attenuation coefficients are those of [33c]. All calculations were performed using the *SHELXL97* [36] program.

Table 3. *Crystallographic Data of Compound* (*S*,*S*,*S*)-**14**.

Crystallized from	$MeOH/CHCl_3/Et_2O$
Empirical formula	$C_{27}H_{36}BrN_3O_6$
Formula weight [g mol^{-1}]	578.50
Crystal color, habit	colorless, plate
Crystal dimensions [mm]	0.03 × 0.15 × 0.22
Temperature [K]	160(1)
Crystal system	monoclinic
Space group	$P2_1$
Z	4
Reflections for cell determination	84590
2θ range for cell determination [°]	4 – 50
Unit cell parameters a [Å]	15.2292(6)
b [Å]	10.1998(4)
c [Å]	18.6076(8)
β [°]	91.810(2)
V [Å^3]	2889.0(2)
D_x [g cm^{-3}]	1.330
μ(MoK_α) [mm^{-1}]	1.470
Scan type	ω
$2\theta_{(max)}$ [°]	50
Transmission factors (min; max)	0.737; 0.982
Total reflections measured	43528
Symmetry independent reflections	10174
Reflections with $I > 2\sigma(I)$	6247
Reflections used in refinement	10163
Parameters refined; restraints	693; 1
Final $R(F)$ [$I > 2\sigma(I)$ reflections]	0.0646
$wR(F^2)$ (all data)	0.1480
Weights: $w = [\sigma^2(F_o^2) + (0.0518P)^2 + 1.5382P]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$	
Goodness of fit	1.036
Final Δ_{max}/σ	0.001
$\Delta\rho$ (max; min) [e Å^{-3}]	0.49; -0.44

References

[1] W. F. DeGrado, *Adv. Prot. Chem.* **1988**, *39*, 51.

[2] J. Rizo, L. M. Gierasch, *Annu. Rev. Biochem.* **1992**, *61*, 387.

[3] V. J. Hruby, F. Al-Obeidi, W. Kazmierski, *Biochem. J.* **1990**, *268*, 249.

[4] M. Mutter, *Angew. Chem., Int. Ed.* **1985**, *24*, 639.

[5] H. Heimgartner, *Angew. Chem., Int. Ed.* **1991**, *30*, 238.

[6] J. M. Humphrey, A. R. Chamberlin, *Chem. Rev.* **1997**, *97*, 2243.

[7] P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1986**, *69*, 1153; **1987**, *70*, 354.

[8] N. Pradeille, A. Linden, K. Möhle, O. Zerbe, H. Heimgartner, *Chem. Biodiv.* **2005**, *2*, 1127.

[9] R. T. N. Luykx, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2003**, *86*, 4093.

[10] N. Pradeille, H. Heimgartner, *J. Pept. Sci.* **2003**, *9*, 827.

[11] S. Stamm, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2003**, *86*, 1371.

[12] J. Lehmann, A. Linden, H. Heimgartner, *Tetrahedron* **1998**, *54*, 8721.

[13] C. B. Bucher, H. Heimgartner, *Helv. Chim. Acta* **1996**, *79*, 1903.

[14] W. Altherr, Ph.D. thesis, Universität Zürich, 1994.

[15] P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1990**, *73*, 13.

[16] S. Stamm, H. Heimgartner, *Eur. J. Org. Chem.* **2004**, 3820.

[17] C. K. Johnson, *ORTEPII*, Report ORNL-5138, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1976.

[18] J. Bernstein, R. E. Davis, L. Shimoni, N.-L. Chang, *Angew. Chem., Int. Ed.* **1995**, *34*, 1555.

[19] Performed by *C.A.T. GmbH & Co.*, Tübingen, Germany.

[20] I. Dannecker-Dörig, Ph.D. thesis, Universität Zürich, 1995.

[21] S. A. Stoykova, Ph.D. thesis, Universität Zürich, 2004.

[22] L. Zhang, C. Goldammer, B. Henkel, F. Zuehl, G. Panhaus, G. Jung, E. Bayer, in 'Innovation Perspect. Solid Phase Synth. Collect. Pap.', 3rd Int. Symp. (1993), 1994, p. 711.

[23] J. M. Villalgordo, H. Heimgartner, *Tetrahedron* **1993**, *49*, 7215.

[24] J. M. Villalgordo, H. Heimgartner, *Helv. Chim. Acta* **1993**, *76*, 2830.

[25] C. Strässler, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **1997**, *80*, 1528.

[26] C. B. Bucher, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **1995**, *78*, 935.

[27] R. Hooft, *KappaCCD Collect Software*, Nonius BV, Delft, The Netherlands, 1999.

[28] Z. Otwinowski, W. Minor, in 'Methods in Enzymology', Vol. 276, 'Macromolecular Crystallography', Part A, Eds. C.W. Carter Jr., R.M. Sweet, Academic Press, New York, 1997, p. 307.

[29] R. H. Blessing, *Acta Crystallogr., Sect A* **1995**, *51*, 33.

[30] G. M. Sheldrick, *SHELXS97, Program for the Solution of Crystal Structures*, University of Göttingen, Germany, 1997.

[31] A. L. Spek, *PLATON*, *Program for the Analysis of Molecular Geometry*, University of Utrecht, The Netherlands, 2005.

[32] a) H. D. Flack, G. Bernardinelli, *Acta Crystallogr., Sect A* **1999**, *55*, 908; b) H. D. Flack, G. Bernardinelli, *J. Appl. Crystallogr.* **2000**, *33*, 1143.

[33] a) E. N. Maslen, A. G. Fox, M. A. O'Keefe, in 'International Tables for Crystallography', Ed. A. J. C. Wilson, Kluwer Academic Publishers, Dordrecht, 1992, Vol. C, Table 6.1.1.1, p. 477; b) D. C. Creagh, W. J. McAuley, *ibid.* Table 4.2.6.8, p. 219; c) D. C. Creagh, J. H. Hubbell, *ibid.* Table 4.2.4.3, p. 200.

[34] R. F. Stewart, E. R. Davidson, W. T. Simpson, *J. Chem. Phys.* **1965**, *42*, 3175.

[35] J. A. Ibers, W. C. Hamilton, *Acta Crystallogr.* **1964**, *17*, 781.

[36] G. M. Sheldrick, *SHELXL97, Program for the Refinement of Crystal Structures*, University of Göttingen, Germany, 1997.

6. Novel *N*-(2,2-Dimethyl-2*H*-azirin-3-yl)-L-prolinates as Aib-Pro Synthons [1]

The syntheses of phenacyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate and allyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate are reported. Reactions of these 2*H*-azirin-3-amines with Z-protected amino acids have shown them to be suitable synthons for the Aib-Pro unit in peptide synthesis. After incorporation into the peptide by means of the 'azirine/oxazolone method', the C-termini of the resulting peptides were deprotected selectively with Zn in AcOH or with a mild Pd^0-promoted procedure, respectively.

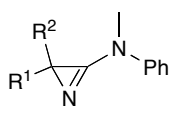
1. Introduction

Peptides that contain α,α-disubstituted α-amino acids are of interest because the rigidity of their peptide backbones leads to a stabilization or even a promotion of secondary structures, such as β-turns or helices [1–4]. Moreover, a group of peptide antibiotics, the peptaibols, contain a high proportion of α,α-disubstituted α-amino acids, in particular α-aminoisobutyric acid (Aib) [5]. A valuable and convenient method for the introduction of these sterically demanding α,α-disubstituted α-amino acids is the 'azirine/oxazolone method' [6–8]. Thus, the reaction of 2*H*-azirin-3-amines **1**, which represent the amino acid synthons, with amino or peptide acids leads to peptide amides, the terminal amide bonds of which can be hydrolyzed selectively to give the extended peptide acids. This method has been applied successfully in the introduction of a variety of α,α-disubstituted α-amino acids into peptides, and it has found application in the synthesis of some peptaibols or segments thereof [9–18].

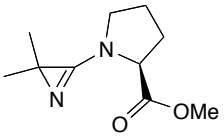
Recently, we adapted the 'azirine/oxazolone method' to solid-phase conditions, in order to additionally profit from their benefits [19]. Moreover, it was shown that also on solid phase the method is not limited to the Aib synthon **1a**, and it was extended successfully to the 1-aminocyclopentane-1-carboxylic acid synthon (**1b**), the 4-amino-3,4,5,6-tetrahydro-2*H*-pyran-4-carboxylic acid synthon (**1c**), and the α-methylphenylalanine synthon (**1d**) [20].

[1] S. Stamm, H. Heimgartner, *Helv. Chim. Acta*, submitted.

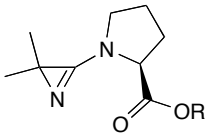
Since the Aib-Pro motif is widespread in peptaibols – in fact, 266 out of the 309 so far known peptaibol sequences contain the Aib-Pro unit [21] – it was of great interest to introduce this unit directly. In solution-phase chemistry, the introduction of the Aib-Pro unit was accomplished with the use of dipeptide synthon **2** [22]. Unexpectedly, its use on solid phase was not successful due to the incompatibility of the linker and the strong basic media which is required for the saponification of the methyl ester [23]. In the course of working out a new strategy for the introduction of the Aib-Pro motif on solid phase, a 2*H*-azirin-3-amine with an easily removable carboxy-protecting group was required.



1a $R^1 = R^2 = Me$
b $R^1 – R^2 = –(CH_2)_4–$
c $R^1 – R^2 = –(CH_2)_2O(CH_2)_2–$
d $R^1 = Me$, $R^2 = PhCH_2$



2



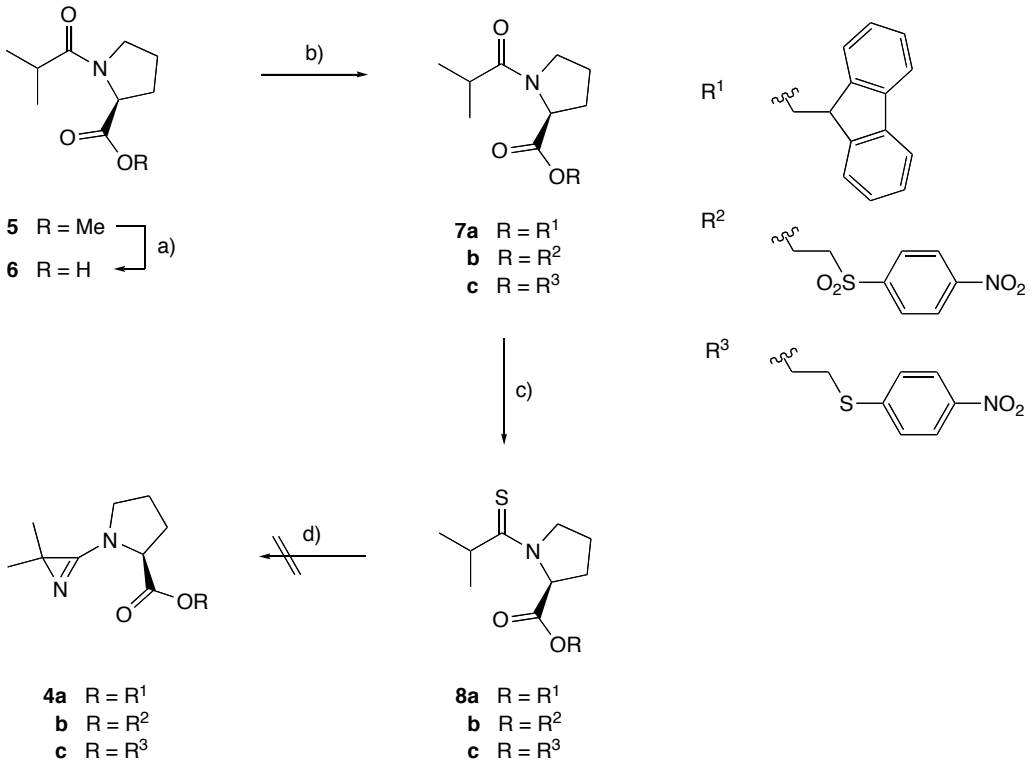
3a $R = PhCOCH_2$
b $R = CH_2CHCH_2$

Herein, we present the synthesis, chemical characterization, and briefly, the use in solution-phase peptide synthesis of the novel Aib-Pro synthons **3a** and **3b**. After introduction into the peptide, the carboxy termini of the resulting extended peptides can be deprotected with Zn in AcOH or with a mild Pd^0-promoted procedure, respectively.

2. Results and Discussion.

Since the synthesis of 2*H*-azirin-3-amines **1** is performed under acidic conditions, the range of carboxy-protecting groups is limited to those which are base labile, which can be removed by reduction, or which are labile towards transition metal complexes, but feature a good stability towards acids. The fluorenylmethyl (Fm) [24][25] and 2-(4-nitrophenylsulfonyl)ethyl [26] protecting groups, which can be cleaved by treatment with secondary amines, should fulfill these prerequisites. Hence, we aimed at the synthesis of the 2*H*-azirin-3-amines **4a** and **4b** as outlined in *Scheme 1*. After saponification of methyl prolinate **5** with LiOH and subsequent carbodiimide induced coupling of acid **6** with the corresponding alcohols, the obtained amides **7a** and **7b** were converted to the thioamides **8a**

and **8b**, respectively, by thionation with *Lawesson* reagent. A slightly modified[2]) procedure compared to that of *Wipf* [27] did not lead to the desired 2*H*-azirin-3-amines **4a** and **4b**. Consequently, we headed for **4b** *via* the 2-(4-nitrophenylsulfanyl)ethyl protected 2*H*-azirin-3-amine **4c**. This route comprises the 'safety catch principle' since the activation for the *β*-elimination is realized after the azirine synthesis by an oxidation of the sulfanyl group[3]). However, the synthesis of **4c** failed too.

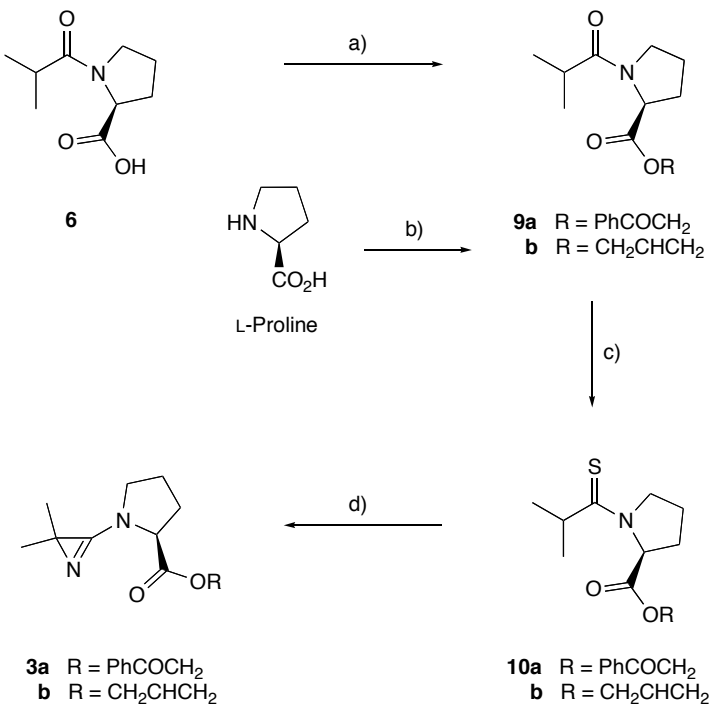


Scheme 1. a) LiOH · H_2O, THF, MeOH, H_2O, r.t.; b) for **7a**: 9*H*-fluoren-9-ylmethanol, DCC, PPY, CH_2Cl_2, r.t.; for **7b**: 2-(4-nitrophenylsulfonyl)ethanol, EDCI, DMAP, CH_2Cl_2, r.t.; for **7c**: 2-(4-nitrophenylsulfanyl)ethanol, DCC, PPY, CH_2Cl_2, r.t.; c) *Lawesson* reagent, MePh, *ca*. 90°; d) 1. $COCl_2$, MePh, CH_2Cl_2, DMF (cat.), 0°; 2. for **4a** and **4b**: $EtN(iPr)_2$, THF, r.t.; for **4c**: DABCO, THF, r.t.; 3. NaN_3, THF, (DMF), r.t. For abbreviations see Experimental Part, General.

[2]) In order to prevent early *β*-elimination, $EtN(iPr)_2$ was used instead of DABCO.

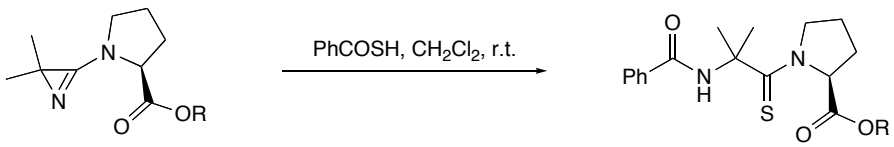
[3]) A preliminary test showed that **2** is stable under mild oxidative conditions (TPAP, NMO).

The phenacyl and allyl ester protecting groups, which can be removed by treatment with Zn/AcOH [28] and $Pd(Ph_3P)_4/PhSiH_3$ [29], respectively, were the next promising candidates for proline protection. The synthesis of the phenacyl and allyl ester protected dipeptide synthons **3a** and **3b** started with the preparation of the esters **9a** and **9b** by alkylation of **6** with phenacyl bromide and allyl bromide, respectively (*Scheme 2*). A direct access from L-proline was accomplished for **9b**. For this purpose, L-proline was esterified with allyl alcohol and then acylated with isobutyryl chloride. After thionation of the amides **9a** and **9b** with *Lawesson* reagent, the synthesis of **3a** and **3b** was achieved by consecutive treatment of the obtained thioamides **10a** and **10b** and catalytic amounts of DMF in CH_2Cl_2 with $COCl_2$, evaporation of the solvent, addition of THF and 1,4-diazabicyclo[2.2.2]octane (DABCO), filtration, and treatment with NaN_3 (*cf.* [27]). After chromatographic workup, the 2*H*-azirin-3-amines **3a** and **3b** were obtained in 58 and 62% yield, respectively, as pale yellow oils.



Scheme 2. a) for **9a**: phenacyl bromide, Et_3N, AcOEt, r.t.; for **9b**: allyl bromide, aliquat 336, CH_2Cl_2, $NaHCO_3$, H_2O, 0°→r.t.; b) 1. $SOCl_2$, allyl alcohol, –20°→65°; 2. isobutyryl chloride, Et_3N, AcOEt, 0°→r.t.; c) *Lawesson* reagent, MePh, *ca.* 90°; d) 1. $COCl_2$, MePh, CH_2Cl_2, DMF (cat.), 0°; 2. DABCO, THF, r.t.; 3. NaN_3, THF, r.t. For abbreviations see Experimental Part, General.

For a chemical characterization and for the examination of the reactivity of the novel 2*H*-azirin-3-amines **3a** and **3b**, they were treated with thiobenzoic acid (*Scheme 3*). The reactions proceeded smoothly and the thiopeptides **11** were obtained in high yield (93 and 94%, resp.). In the case of **11a**, crystals suitable for an X-ray crystal-structure determination were obtained (*Figure*). Compound **11a** in the crystal is enantiomerically pure and the absolute configuration of the molecule has been determined independently by the diffraction experiment. The molecule has the expected *S*-configuration. The amide group forms an intermolecular H-bond with the S-atom of an adjacent molecule and thereby links the molecules into extended chains, which run parallel to the [0 1 0] direction and can be described by the graph set motif [30] of C(5).



3a R = $PhCOCH_2$
b R = CH_2CHCH_2

11a R = $PhCOCH_2$
b R = CH_2CHCH_2

Scheme 3

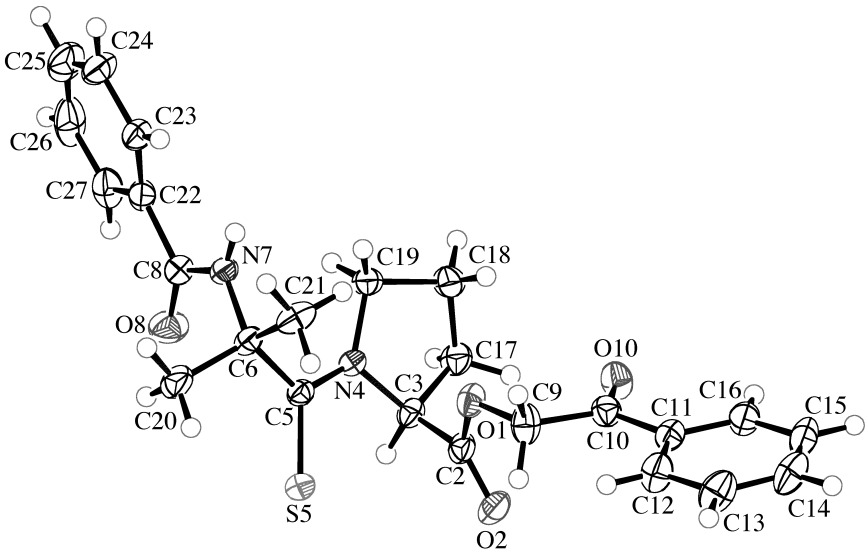
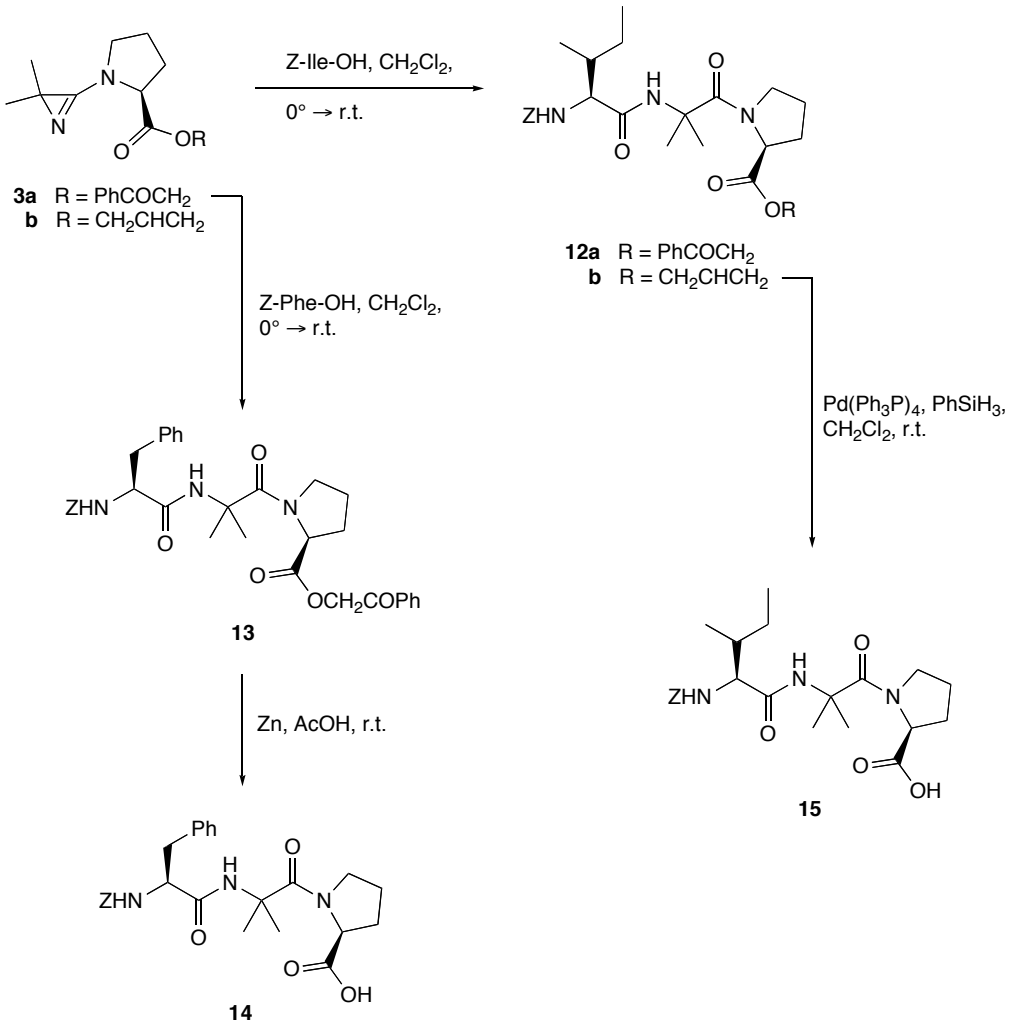


Fig. *ORTEP Plot* [31] *of the molecular structure of* **11a** (50% probability ellipsoids; arbitrary numbering of atoms).

In order to examine the use of **3a** and **3b** in peptide synthesis, reactions with N-protected amino acids were performed. The Aib-Pro synthon **3a** was reacted with Z-Ile-OH and Z-Phe-OH to give the tripeptides **12a** and **13**, respectively, while the reaction of **3b** with Z-Ile-OH yielded the tripeptide **12b** (*Scheme 4*). All reactions gave the products in high purity and in very good yields (90–94%, after chromatographic workup). Then, the selective deprotection of the C-terminus of **13** was accomplished with Zn-powder in AcOH, and yielded peptide acid **14**. Deprotection of the phenacyl esters with $Bu_4N^+F^-$ in DMF [32] would possibly be transferable to solid-phase conditions, which is the ultimate use of the new azirine. Therefore, **12a** and **13** were subjected to $Bu_4N^+F^-$ (3 equiv.) in DMF and THF, respectively, but in none of the experiments a peptide acid **14** or **15**, respectively, could be isolated.



Scheme 4

The deprotection of the C-terminus can be performed under milder conditions if synthon **3b** is used in the peptide chain extension. This was illustrated with the model peptide **12b**, in which the allyl ester group was smoothly removed by treatment with $Pd(Ph_3P)_4$ and $PhSiH_3$ in CH_2Cl_2 to give peptide acid **15**. Moreover, these deprotecting conditions should be applicable on solid phase.

3. Conclusions

The novel 2*H*-azirin-3-amines **3a** and **3b**, which contain a phenacyl ester and an allyl ester, respectively, as carboxy-protecting group, have been synthesized. These azirines represent Aib-Pro synthons, and this dipeptide unit can be introduced conveniently into peptides by the 'azirine/oxazolone method'. The C-terminus of the resulting peptide esters can be deprotected under non-basic conditions, *i.e.* by treatment with Zn/AcOH and $Pd(Ph_3P)_4/PhSiH_3$. The use of **3a** and **3b** as building blocks for the 'azirine/oxazolone method' on solid phase are in progress.

Experimental Part

1. *General.* – Reagents were obtained from commercial suppliers and were used without further purification. Solvents were purified by standard procedures. Compound **5** was prepared according to [22]. TLC: *Merck* TLC aluminum sheets, silica gel *60* F_{254}. Prep. TLC: *Merck* PLC plates (glass), silica gel *60* F_{254}. Column chromatography (CC): *Uetikon-Chemie*, silica gel *C-560*. M.p.: *Büchi Melting Point B-450* apparatus, uncorrected. IR Spectra: *Perkin-Elmer*, *Spectrum One FT-IR* spectrophotometer; unless otherwise stated in KBr, absorptions in cm^{-1}. NMR Spectra: *Bruker ARX-300*, *Bruker AV-600*, or *Bruker AV-700* instruments; in $CDCl_3$, δ in ppm, TMS as internal standard, coupling constants *J* in Hz. 2D-NMR experiments were performed for assignment of the signals. In ^1H- and ^{13}C-NMR spectra where two conformers were observed, only the signals of the major conformer are shown. The ratio of the two conformers is given in parentheses. MS (*m*/*z* (rel.%)): *Bruker ESQUIRE-LC* quadrupole ion trap instrument. *Abbreviations:* Aib: α-aminoisobutyric acid; DABCO: 1,4-diazabicyclo[2.2.2]octane; DCC: *N*,*N*'-dicyclohexylcarbodiimid; DMAP: 4-(*N*,*N*-dimethyl-

amino)pyridine; EDCI: *N*-ethyl-*N*'-[3-(dimethylamino)propyl]carbodiimide hydrochloride; PPY: 4-pyrrolidinopyridine.

2. *Synthesis of 2*H-*Azirin-3-amines* **3a** *and* **3b**. N-*(2-Methylpropanoyl)*-L-*proline* (**6**). A soln. of methyl *N*-(2-methylpropanoyl)-L-prolinate (**5**) (2.000 g, 10.04 mmol) and $LiOH \cdot H_2O$ (0.843 g, 20.11 mmol) in THF/MeOH/H_2O (3:1:1; 100 ml) was stirred at r.t. overnight. The org. solvents were removed under reduced pressure and the residue was washed with Et_2O. At 0°, the aq. phase was acidified with 1M HCl and saturated with NaCl. After extraction with CH_2Cl_2, the combined org. layers were washed with brine, dried ($MgSO_4$), and concentrated *i.v.* to give **6** (1.775 g, 96%). Colorless powder. M.p. 122.6–123.7°. IR: 2969*s*, 2942*s*, 2879*s*, 2771*m*, 2606*m*, 1721v*s*, 1600v*s*, 1509*w*, 1478*s*, 1446v*s*, 1332*m*, 1266*m*, 1254*s*, 1228v*s*, 1191*s*, 1167*m*, 1154*m*, 1092*s*, 1042*w*, 968*w*, 914*m*, 838*w*, 808*w*, 752*w*. ^{1}H-NMR (300 MHz, conformers (95:5)): 10.9–9.6 (br. *s*, CO_2H); 4.61–4.57 (*m*, CH(α)(Pro)); 3.66–3.61, 3.58–3.50 (2*m*, CH_2(δ)(Pro)); 2.72 (*sept.*, *J* = 6.8, Me_2CH); 2.44–2.40, 2.10–1.99 (2*m*, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.53, 1.16 (2*d*, *J* = 6.8, 2 Me). ^{13}C-NMR (75 MHz): 179.1, 172.8 (2*s*, 2 CO); 60.0 (*d*, CH(α)(Pro)); 47.6 (*t*, CH_2(δ)(Pro)); 32.5 (*d*, Me_2CH); 27.4, 24.9 (2*t*, CH_2(β)(Pro), CH_2(γ)(Pro)); 19.0, 18.5 (2*q*, 2 Me). ESI-MS (MeOH): 208 (100, $[M+Na]^+$). Anal. calc. for $C_9H_{15}NO_3$ (185.22): C 58.36, H 8.16, N 7.56; found: C 58.51, H 8.06, N 7.77.

Phenacyl N-*(2-Methylpropanoyl)*-L-*prolinate* (**9a**). A mixture of **6** (0.801 g, 4.32 mmol), Et_3N (601 µl, 4.32 mmol) and phenacyl bromide (0.860g, 4.32 mmol) in AcOEt (20 ml) was stirred at r.t. overnight. The mixture was filtered, and the soln. was concentrated *i.v.* CC (SiO_2, AcOEt/hexane 6:4) yielded **9a** (1.280 g, 98%). Colorless oil. IR (film): 3488*m*, 3391*w*, 3270*w*, 3063*s*, 2974v*s*, 2935v*s*, 2876v*s*, 1755v*s*, 1701v*s*, 1644v*s*, 1598v*s*, 1582v*s*, 1470v*s*, 1449v*s*, 1426v*s*, 1376v*s*, 1364v*s*, 1318v*s*, 1279v*s*, 1230v*s*, 1173v*s*, 1092v*s*, 1043*s*, 1001v*s*, 973v*s*, 953v*s*, 921*m*, 886*m*, 851*m*, 810*s*, 753v*s*, 735v*s*. ^{1}H-NMR (300 MHz, conformers (85:15)): 7.90–7.87, 7.63–7.57, 7.52–7.45 (3*m*, 5 arom. H); 5.55, 5.21 (*AB*, *J* = 16.5, CH_2CO); 4.68–4.62 (*m*, CH(α)(Pro)); 3.76–3.67, 3.62–3.54 (2*m*, CH_2(δ)(Pro)); 2.69 (*sept.*, *J* = 6.8, Me_2CH); 2.42–2.13, 2.10–1.97 (2*m*, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.15, 1.14 (2*d*, *J* = 6.8, 2 Me). ^{13}C-NMR (75 MHz): 192.2 (*s*, CO(carbonyl)); 175.9 (*s*, CO(amide)); 171.8 (*s*, CO(ester)); 134.1 (*s*, arom. C); 133.8, 128.8, 127.6 (3*d*, 5 arom. CH); 60.0 (*t*, CH_2CO); 58.5 (*d*, CH(α)(Pro)); 46.7 (*t*, CH_2(δ)(Pro)); 32.2 (*d*, Me_2CH); 29.1, 24.8 (2*t*, CH_2(β)(Pro), CH_2(γ)(Pro)); 18.7, 18.6 (2*q*, 2 Me). ESI-MS (MeOH, NaI): 326 (100, $[M+Na]^+$). Anal. calc. for $C_{17}H_{21}NO_4$ (303.35): C 67.31, H 6.98, N 4.62; found: C 67.11, H 6.61, N 4.62.

Allyl N-*(2-Methylpropanoyl)*-L-*prolinate* (**9b**). *From* **6**. A soln. of allyl bromide (22.8 ml, 269.50 mmol) and aliquat 336 (21.6 g) in CH_2Cl_2 (75 ml) was added to a soln. of **6** (10.004 g,

54.01 mmol) and $NaHCO_3$ (4.539 g, 54.03 mmol) in H_2O (75 ml) at 0°. The mixture was vigorously stirred at r.t. for 3 d, then H_2O (50 ml) was added and the suspension was extracted with CH_2Cl_2. The combined org. layers were dried ($MgSO_4$) and concentrated *i.v.* CC (SiO_2, AcOEt/hexane 4:6 → 1:1) yielded **9b** (11.328 g, 93%). Colorless liquid.

From L-*Proline*. At –20°, $SOCl_2$ (1.70 ml, 23.36 mmol) was added to allyl alcohol (12 ml, 176.45 mmol), then L-proline (2.000 g, 17.37 mmol) was added, and the mixture was slowly heated to 65° and stirred at 65° for 1 h. The mixture was concentrated *i.v.*, the residue was dissolved in AcOEt (60 ml), and Et_3N (5.05 ml, 36.23 mmol) and isobutyryl chloride (1.85 ml, 17.52 mmol) were added at 0°. The mixture was stirred at r.t. overnight, H_2O (20 ml) was added, and the mixture was extracted with AcOEt. The combined org. layers were dried ($MgSO_4$) and concentrated *i.v.* CC (AcOEt/hexane 1:1) yielded **9b** (3.164 g, 81%). Colorless liquid. IR (film): 3568*w*, 3479*w*, 3085*w*, 2973v*s*, 2935*s*, 2877*s*, 1745v*s*, 1650v*s*, 1741v*s*, 1426v*s*, 1377*s*, 1362*s*, 1318v*s*, 1274*s*, 1242*s*, 1177v*s*, 1091*s*, 1044*m*, 990*s*, 954*m*, 930*s*, 882*w*, 819*w*, 754*m*. ^{1}H-NMR (300 MHz, conformers (85:15)): 5.97–5.84 (*m*, CH_2=C*H*); 5.37–5.20 (*m*, C*H*$_2$=CH); 4.68–4.60 (*m*, CH_2O); 4.53–4.48 (*m*, CH(α)(Pro)); 3.73–3.64, 3.62–3.53 (2*m*, CH_2(δ)(Pro)); 2.68 (*sept.*, *J* = 6.8, Me_2C*H*); 2.29–1.90 (*m*, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.16, 1.13 (2*d*, *J* = 6.8, 2 Me). ^{13}C-NMR (75 MHz): 176.0 (*s*, CO(amide)); 172.3 (*s*, CO(ester)); 132.2 (*d*, CH_2=*C*H); 118.4 (*t*, *C*H$_2$=CH); 65.7 (*t*, CH_2O); 58.9 (*d*, CH(α)(Pro)); 46.9 (*t*, CH_2(δ)(Pro)); 32.4 (*d*, Me_2*C*H); 29.3, 25.1 (2*t*, CH_2(β)(Pro), CH_2(γ)(Pro)); 19.0, 18.8 (2*q*, 2 Me). ESI-MS (MeOH): 298 (13, [*M*+Na+MeOH+H_2O]$^+$), 248 (100, [*M*+Na]$^+$). Anal. calc. for $C_{12}H_{19}NO_3$ (225.28): C 63.98, H 8.50, N 6.22; found: C 63.71, H 8.45, N 6.13.

Phenacyl N-*(2-Methylpropanthioyl)*-L-*prolinate* (**10a**). A suspension of *Lawesson* reagent (dried *i.v.*, 444 mg, 1.10 mmol) and **9a** (600 mg, 1.98 mmol) in toluene (20 ml) was heated at 90° (oilbath) for 1 h. After cooling to r.t., the mixture was filtered, and the solvent was evaporated. CC (SiO_2, CH_2Cl_2/MeOH 200:1 → 100:1) yielded **10a** (571 mg, 90%). Colorless solid. M.p. 83.9–84.9°. IR: 2976*m*, 2946*w*, 2928*w*, 2868*w*, 1749v*s*, 1701v*s*, 1596*m*, 1579*w*, 1468*s*, 1445v*s*, 1419*m*, 1373*m*, 1356*m*, 1345*m*, 1309*w*, 1263*m*, 1255*m*, 1237*v*, 1192v*s*, 1173*s*, 1156*s*, 1130*m*, 1013*s*, 1000*w*, 967*m*, 910*w*, 815*w*, 757*m*, 735*w*, 690*s*. ^{1}H-NMR (600 MHz, conformers (87:13)): 7.90–7.88, 7.65–7.59, 7.52–7.47 (3*m*, 5 arom. H); 5.54, 5.25 (*AB*, *J* = 16.4, CH_2CO); 5.27–5.25 (*m*, CH(α)(Pro)); 3.97–3.93, 3.79–3.75 (2*m*, CH_2(δ)(Pro)); 3.07 (*sept.*, *J* = 6.6, Me_2C*H*); 2.56–2.50, 2.40–2.31, 2.21–2.14 (3*m*, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.26, 1.23 (2*d*, *J* = 6.6, 2 Me). ^{13}C-NMR (150 MHz): 209.9 (*s*, CS); 192.2 (*s*, CO(carbonyl)); 170.0 (*s*, CO(ester)); 134.1 (*s*, 1 arom. C); 134.0, 128.9, 127.7

(3*d*, 5 arom. CH); 66.3 (*t*, *C*H_2CO); 65.2 (*d*, CH(*α*)(Pro)); 50.4 (*t*, CH_2(*δ*)(Pro)); 38.7 (*d*, Me_2*C*H); 29.0, 24.7 (2*t*, CH_2(*β*)(Pro), CH_2(*γ*)(Pro)); 22.7, 22.4 (2*q*, 2 Me). ESI-MS (MeOH, NaI): 342 (100, [*M*+Na]$^+$). Anal. calc. for $C_{17}H_{21}NO_3S$ (319.42): C 63.92, H 6.63, N 4.39, S 10.04; found: C 64.07, H 6.37, N 4.30, S 10.08.

Allyl N-*(2-Methylpropanthioyl)*-L-*prolinate* (**10b**). A suspension of *Lawesson* reagent (dried *i.v.*, 5.584 g, 13.81 mmol) and **9b** (5.618 g, 24.94 mmol) in toluene (70 ml) was heated at 95° (oilbath) for 1 h. After cooling to r.t., the mixture was filtered, and the solvent was evaporated. CC (SiO_2, CH_2Cl_2/MeOH 400:1 → 100:1) yielded **10b** (5.439 g, 90%). Pale yellow oil. IR (film): 3084*w*, 2973v*s*, 2931*s*, 2878*s*, 1742v*s*, 1648*w*, 1440v*s*, 1381*s*, 1360*s*, 1333v*s*, 1268v*s*, 1227v*s*, 1191v*s*, 1170v*s*, 1126*s*, 1088*m*, 1046*m*, 1016v*s*, 989*s*, 969*s*, 930*s*, 874*m*, 784*w*. ^{1}H-NMR (700 MHz, conformers (83:17)): 5.94–5.88 (*m*, CH_2=C*H*); 5.37–5.23 (*m*, CH_2=CH); 5.13–5.11 (*m*, CH(*α*)(Pro)); 4.67–4.60 (*m*, CH_2O); 3.93–3.88, 3.78–3.74 (2*m*, CH_2(*δ*)(Pro)); 3.05 (*sept.*, *J* = 6.6, Me_2C*H*); 2.30–2.25, 2.23–2.17, 2.14–2.09 (3*m*, CH_2(*β*)(Pro), CH_2(*γ*)(Pro)); 1.25, 1.24 (2*d*, *J* = 6.6, 2 Me). ^{13}C-NMR (175 MHz): 209.9 (*s*, CS); 170.5 (*s*, CO); 132.1 (*d*, CH_2=*C*H); 118.7 (*t*, *C*H_2=CH); 66.0 (*t*, CH_2O); 65.4 (*d*, CH(*α*)(Pro)); 50.4 (*t*, CH_2(*δ*)(Pro)); 38.9 (*d*, Me_2*C*H); 29.0, 24.9 (2*t*, CH_2(*β*)(Pro), CH_2(*γ*)(Pro)); 22.9, 22.5 (2*q*, 2 Me). ESI-MS (MeOH): 264 (100, [*M*+Na]$^+$). Anal. calc. for $C_{12}H_{19}NO_2S$ (241.35): C 59.72, H 7.93, N 5.80, S 13.29; found: C 59.80, H 7.65, N 5.58, S 13.07.

Phenacyl N-*(2,2-Dimethyl-2*H-*azirin-3-yl)*-L-*prolinate* (**3a**). A soln. of $COCl_2$ in toluene (20%, 1.8 ml, 3.47 mmol) was added to a soln. of **10a** (1.001 g, 3.13 mmol) and 3 drops of DMF in CH_2Cl_2 (10 ml) at 0°, the mixture was stirred for 30 min at 0°, and the volatiles were removed *i.v.* THF (15 ml) and DABCO (0.351 g, 3.13 mmol) were added under vigorous stirring to the residue, and the mixture was stirred at r.t. for 30 min. The solid was removed by filtration under N_2 and washed with THF. To the filtrate, NaN_3 (0.617 g, 9.49 mmol) was added, and the resulting mixture was stirred at r.t. for 4 d. After addition of Et_2O, the resulting suspension was filtered over a *Celite* pad, and the solvent was removed *i.v.* CC (SiO_2, AcOEt/hexane 6:4, Et_3N (1%)) yielded **3a** (0.542 g, 58%). Pale yellow oil. IR (film): 3434*w*, 3388*w*, 3063*w*, 2976*s*, 2942*s*, 2879*m*, 1771v*s*, 1703v*s*, 1598*m*, 1581*w*, 1450*s*, 1417*m*, 1369*s*, 1345*m*, 1277*s*, 1233v*s*, 1174v*s*, 1095*m*, 1001*w*, 964*s*, 911*w*, 846*w*, 819*w*, 758*m*, 690*s*. ^{1}H-NMR (300 MHz): 7.91–7.88, 7.65–7.59, 7.52–7.47 (3*m*, 5 arom. H); 5.47–5.32 (*m*, CH_2CO); 4.50 (br. *s*, CH(*α*)(Pro)); 3.74–3.67, 3.62–3.54 (2*m*, CH_2(*δ*)(Pro)); 2.50–2.04 (*m*, CH_2(*β*)(Pro), CH_2(*γ*)(Pro)); 1.35, 1.31 (2*s*, 2 Me). ^{13}C-NMR (75 MHz): 191.5 (*s*, CO(carbonyl)); 171.7 (*s*, CO(ester)); 165.5 (*s*, C(3)); 134.1 (*d*, 1 arom. CH); 134.0 (*s*, 1 arom. C); 129.0, 127.7 (2*d*, 4

arom. CH); 66.4 (*t*, *C*H_2CO); *ca*. 60.9 (br. *d*, CH(*α*)(Pro)); *ca*. 46.9 (br. *t*, CH_2(*δ*)(Pro)); 39.2 (*s*, C(2)); 30.6 (*t*, CH_2(*β*)(Pro)); 25.2 (*q*, 2 Me); 24.0 (*t*, CH_2(*γ*)(Pro)). ESI-MS (MeOH): 323 (100, $[M+Na]^+$). Anal. calc. for $C_{17}H_{20}N_2O_3$ (300.35): C 67.98, H 6.71, N 9.33; found: C 67.73, H 6.53, N 9.35.

Allyl N-*(2,2-Dimethyl-2*H*-azirin-3-yl)*-L-*prolinate* (**3b**). A soln. of $COCl_2$ in toluene (20%, 2.6 ml, 5.02 mmol) was added to a soln. of **10b** (1.008 g, 4.14 mmol) and 3 drops of DMF in CH_2Cl_2 (10 ml) at 0°, the mixture was stirred for 30 min at 0°, and the volatiles were removed *i.v*. The residue was dissolved in THF (15 ml), DABCO (0.465 g, 4.15 mmol) was added, and the mixture was stirred at r.t. for 30 min. The solid was removed by filtration under N_2 and washed with THF. To the filtrate, NaN_3 (0.810 g, 12.46 mmol) was added, and the resulting mixture was stirred at r.t. for 4 d. After addition of Et_2O, the resulting suspension was filtered over a *Celite* pad, and the solvent was removed *i.v*. CC (SiO_2, AcOEt/hexane 6:4, Et_3N (1%)) yielded **3b** (0.575 g, 63%). Pale yellow oil. IR (film): 3458*w*, 3087*w*, 2976*s*, 2943*s*, 2879*s*, 1770v*s*, 1745v*s*, 1649*w*, 1454*s*, 1413*m*, 1368*s*, 1352*m*, 1272*s*, 1235*s*, 1177v*s*, 1093*m*, 1043*w*, 1015*m*, 987*s*, 933*m*. ^{1}H-NMR (300 MHz): 5.97–5.84 (*m*, CH_2=C*H*); 5.36–5.24 (*m*, C*H*$_2$=CH); 4.67–4.61 (*m*, CH_2O); 4.35–4.34 (*m*, CH(*α*)(Pro)); 3.70–3.52 (*m*, CH_2(*δ*)(Pro)); 2.39–1.99 (*m*, CH_2(*β*)(Pro), CH_2(*γ*)(Pro)); 1.32, 1.29 (2*s*, 2 Me). ^{13}C-NMR (75 MHz): 171.6 (*s*, CO); 165.2 (*s*, C(3)); 131.4 (*d*, CH_2=*C*H); 118.8 (*t*, *C*H_2=CH); 65.8 (*t*, CH_2O); *ca*. 60.8 (br. *d*, CH_2(*δ*)(Pro)); *ca*. 46.9 (br. *t*, CH_2(*δ*)(Pro)); 39.0 (*s*, C(2)); 30.2 (*t*, CH_2(*β*)(Pro)); 25.0 (*q*, 2 Me); 23.9 (*t*, CH_2(*γ*)(Pro)). ESI-MS (MeOH): 255 (23, $[M+H+MeOH]^+$), 245 (100, $[M+Na]^+$), 223 (29, $[M+H]^+$), 196 (25). Anal. calc. for $C_{12}H_{18}N_2O_2 \cdot 0.2\ H_2O$ (225.88): C 63.81, H 8.21, N 12.40; found: C 63.85, H 8.12, N 12.32.

3. *Reactions of 2*H*-Azirin-3-amines* **3a** *and* **3b** *with* PhCOSH. *Phenacyl* N-*[2-(Benzoylamino)-2-methyl-1-thioxopropyl]*-L-*prolinate* (**11a**). A soln. of PhCOSH (20 mg, 0.145 mmol) in CH_2Cl_2 (2 ml) was added to a soln. of **3a** (40 mg, 0.133 mmol) in CH_2Cl_2 (2.5 ml) at 0°. The mixture was stirred at r.t. overnight, the solvent was evaporated, and the crude product was purified by prep. TLC (CH_2Cl_2/MeOH 50:1, 2 × dev.; CH_2Cl_2/MeOH 30:1, 1 × dev.) to give **11a** (54 mg, 93%). Colorless crystals. M.p. 189.1–190.2°. IR: 3361*m*, 3301*m*, 3059*w*, 2984*m*, 2932*m*, 2881*w*, 1749v*s*, 1702v*s*, 1642v*s*, 1598*s*, 1579*s*, 1524v*s*, 1486*s*, 1449v*s*, 1419v*s*, 1385*s*, 1362*s*, 1341*m*, 1301*s*, 1288*s*, 1264*s*, 1226v*s*, 1186v*s*, 1160v*s*, 1092*m*, 1074*m*, 1043*s*, 1028*m*, 1001*m*, 965*s*, 911*w*, 884*w*, 803*w*, 754*s*, 720*s*, 690v*s*. ^{1}H-NMR (600 MHz): 8.51 (*s*, NH); 7.90–7.86, 7.63–7.60, 7.51–7.48, 7.45–7.43 (4*m*, 10 arom. H); 5.53, 5.27 (*AB*, *J* = 16.4, CH_2CO); 5.38–5.36 (*m*, CH(*α*)(Pro)); 4.12–4.08, 3.96–3.92 (2*m*, CH_2(*δ*)(Pro)); 2.51–2.48, 2.39–2.31, 2.14–2.10 (3*m*, CH_2(*β*)(Pro), CH_2(*γ*)(Pro)); 1.95, 1.89 (2*s*, 2 Me). ^{13}C-

NMR (150 MHz): 205.9 (*s*, CS); 192.1 (*s*, CO(carbonyl)); 169.8 (*s*, CO(ester)); 164.9 (*s*, CO(amide)); 135.0 (*s*, 1 arom. C); 134.0 (*d*, 1 arom. CH); 134.0 (*s*, 1 arom. C); 131.4, 128.9, 128.5, 127.7, 127.0 (5*d*, 9 arom. CH); 69.1 (*d*, CH(α)(Pro)); 66.3 (*t*, CH_2CO); 61.2 (*s*, C(α)(Aib)); 53.6 (*t*, CH_2(δ)(Pro)); 28.0 (*t*, CH_2(β)(Pro)); 26.0 (*t*, CH_2(γ)(Pro)); 25.8, 24.9 (2*q*, 2 Me). ESI-MS (MeOH): 461 (100, $[M+Na]^+$). Anal. calc. for $C_{24}H_{26}N_2O_4S$ (438.54): C 65.73, H 5.98, N 6.39, S 7.31; found: C 65.33, H 5.72, N 6.24, S 7.02. Suitable crystals for the X-ray crystal-structure determination were grown from $CDCl_3/Et_2O$.

Allyl N-*[2-(Benzoylamino)-2-methyl-1-thioxopropyl]*-L-*prolinate* (**11b**). A soln. of PhCOSH (27 mg, 0.195 mmol) in CH_2Cl_2 (3 ml) was added to a soln. of **3b** (40 mg, 0.180 mmol) in CH_2Cl_2 (2 ml) at 0°. The mixture was stirred at r.t. overnight, the solvent was evaporated, and the crude product was purified by prep. TLC (CH_2Cl_2/MeOH 40:1, Et_3N (0.5%), 2 × dev.) to give **11b** (61 mg, 94%). Colorless powder. M.p. 46.5–48.0°. IR: 3303*m*, 3059*w*, 2984*m*, 2925*s*, 2880*m*, 2853*m*, 1741v*s*, 1639v*s*, 1602*m*, 1578*m*, 1530v*s*, 1488*s*, 1461*s*, 1419v*s*, 1385*s*, 1362*s*, 1340*m*, 1301*s*, 1292*s*, 1271*s*, 1245*m*, 1187v*s*, 1160v*s*, 1086*w*, 1075*w*, 1046*s*, 1028*w*, 988*m*, 969*m*, 931*m*, 885*w*, 803*w*, 717*s*, 694*m*. ^{1}H-NMR (300 MHz): 8.66 (br. *s*, NH); 7.89–7.85, 7.52–7.40 (2*m*, 5 arom. H); 5.99–5.86 (*m*, CH_2=C*H*); 5.39–5.20 (*m*, CH_2=CH, CH(α)(Pro)); 4.65–4.63 (*m*, CH_2O); 4.08–3.91 (*m*, CH_2(δ)(Pro)); 2.34–2.15, 2.10–2.00 (2*m*, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.95, 1.89 (2*s*, 2 Me). ^{13}C-NMR (75 MHz): 205.8 (*s*, CS); 170.0, 164.7 (2*s*, 2 CO); 135.1 (*s*, 1 arom. C); 131.7, 131.2, 128.4, 126.9 (4*d*, 5 arom. CH, CH_2=*C*H); 118.6 (*t*, *C*H_2=CH); 68.9 (*d*, CH(α)(Pro)); 65.8 (*t*, CH_2O); 61.0 (*s*, C(α)(Aib)); 53.4 (*t*, CH_2(δ)(Pro)); 27.8 (*t*, CH_2(β)(Pro)); 25.8 (*t*, CH_2(γ)(Pro)); 25.5, 24.7 (2*q*, 2 Me(Aib)). ESI-MS (MeOH): 383 (100, $[M+Na]^+$), 367 (17, $[M(S\rightarrow O)+Na]^+$). Anal. calc. for $C_{19}H_{24}N_2O_3S$ (360.47): C 63.31, H 6.71, N 7.77, S 8.90; found: C 63.25, H 6.45, N 7.68, S 8.67.

4. *Synthesis of Model Peptides. Phenacyl* N-*{2-[(2(*S*),3(*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-methyl-1-oxopentyl)amino]-2-methyl-1-oxopropyl}*-L-*prolinate* (**12a**). A soln. of **3a** (65 mg, 0.216 mmol) in CH_2Cl_2 (2.5 ml) was added to a soln. of Z-Ile-OH (57 mg, 0.215 mmol) in CH_2Cl_2 (2.5 ml) at 0°. The mixture was stirred at r.t. overnight, the solvent was evaporated, and the crude product was purified by CC (SiO_2, CH_2Cl_2/MeOH 60:1) to give **12a** (105 mg, 86%). Colorless solid. M.p. 70.1–71.5°. IR: 3318*s*, 3064*w*, 3034*w*, 2966*s*, 2936*m*, 2877*m*, 1753v*s*, 1702v*s*, 1660v*s*, 1633v*s*, 1599*m*, 1531v*s*, 1468*s*, 1451*s*, 1409v*s*, 1378*s*, 1363*s*, 1286*s*, 1230v*s*, 1167v*s*, 1096*m*, 1042*m*, 1028*m*, 1001*w*, 977*m*, 754*s*, 693*s*. ^{1}H-NMR (600 MHz): 7.89–7.88, 7.62–7.59, 7.50–7.47, 7.37–7.31 (4*m*, 10 arom. H); 6.97 (*s*, NH(Aib)); 5.57, 5.21 (*AB*, J = 16.4, CH_2CO); 5.41 (*d*, J = 8.8, NH(Ile)); 5.12, 5.08 (*AB*, J = 12.3,

CH_2(carbamate)); 4.72–4.69 (*m*, CH(α)(Pro)); 4.00–3.97 (*m*, CH(α)(Ile)); 3.77–3.73, 3.57–3.54 (2*m*, CH_2(δ)(Pro)); 2.32–2.30 (*m*, 1 H of CH_2(β)(Pro)); 2.19–2.10 (*m*, 1 H of CH_2(β)(Pro), 1 H of CH_2(γ)(Pro)); 1.90–1.88 (*m*, 1 H of CH_2(γ)(Pro)); 1.83–1.82 (*m*, CH(β)(Ile)); 1.65, 1.61 (2*s*, 2 Me(Aib)); 1.53–1.48, 1.16–1.11 (2*m*, CH_2(γ)(Ile)); 0.92 (*d*, *J* = 6.8, *Me*CH(β)(Ile)); 0.89 (*t*, *J* = 7.4, *Me*CH_2(γ)(Ile)). ^{13}C-NMR (150 MHz): 192.2 (*s*, CO(carbonyl)); 172.2 (*s*, CO(Aib)); 171.8 (*s*, CO(Pro)); 169.6 (*s*, CO(Ile)); 156.2 (*s*, CO(carbamate)); 136.3, 134.1 (2*s*, 2 arom. C); 134.0, 128.9, 128.5, 128.2, 128.0, 127.7 (6*d*, 10 arom. CH); 66.9 (*t*, Ph*C*H_2); 66.2 (*t*, *C*H_2CO); 60.9 (*d*, CH(α)(Pro)); 59.7 (*d*, CH(α)(Ile)); 57.1 (*s*, C(α)(Aib)); 48.2 (*t*, CH_2(δ)(Pro)); 37.8 (*d*, CH(β)(Ile)); 27.9 (*t*, CH_2(β)(Pro)); 25.8 (*t*, CH_2(γ)(Pro)); 24.9 (*t*, CH_2(γ)(Ile)); 23.6, 23.2 (2*q*, 2 Me(Aib)); 15.4 (*q*, *Me*CH(β)(Ile)); 11.4 (*q*, *Me*CH_2(γ)(Ile)). ESI-MS (MeOH): 588 (100, [*M*+Na]$^+$). Anal. calc. for $C_{31}H_{39}N_3O_7$ (565.66): C 65.82, H 6.95, N 7.43; found: C 65.53, H 7.06, N 7.27.

Allyl N-*{2-[(2(*S*),3(*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-methyl-1-oxopentyl)amino]-2-methyl-1-oxopropyl}*-L-*prolinate* (**12b**). A soln. of **3b** (60 mg, 0.270 mmol) in CH_2Cl_2 (3 ml) was added to a soln. of Z-Ile-OH (79 mg, 0.297 mmol) in CH_2Cl_2 (2 ml) at 0°. The mixture was stirred at r.t. overnight, the solvent was evaporated, and the crude product was purified by CC (SiO_2, CH_2Cl_2/MeOH 20:1) to give **12b** (123 mg, 93%). Colorless powder. M.p. 135.0–136.1°. IR: 3287*s*, 3256*s*, 3065*m*, 2964*s*, 2937*m*, 2876*w*, 1751v*s*, 1715v*s*, 1666v*s*, 1619v*s*, 1547v*s*, 1497*m*, 1453*s*, 1419*s*, 1383*s*, 1366*m*, 1355*m*, 1285*s*, 1272*s*, 1243v*s*, 1208*m*, 1174v*s*, 1162v*s*, 1126*m*, 1095*w*, 1047*m*, 1028*m*, 987*m*, 949*w*, 923*w*, 877*w*, 853*w*, 778*w*, 751*w*, 738*m*, 700*m*. ^{1}H-NMR (600 MHz): 7.37–7.29 (*m*, 5 arom. H); 6.97 (br. *s*, NH(Aib)); 5.94–5.87 (*m*, CH_2=C*H*); 5.40 (*d*, *J* = 8.5, NH(Ile)); 5.35–5.31, 5.25–5.23 (2*m*, CH_2=CH); 5.11, 5.08 (*AB*, *J* = 12.3, CH_2(carbamate)); 4.66–4.58 (*m*, CH_2O); 4.58–4.55 (*m*, CH(α)(Pro)); 4.00–3.97 (*m*, CH(α)(Ile)); 3.72–3.69, 3.58–3.55 (2*m*, CH_2(δ)(Pro)); 2.11–2.07 (*m*, 1 H of CH_2(β)(Pro)); 2.04–1.97 (*m*, 1 H of CH_2(γ)(Pro)); 1.91–1.82 (*m*, 1 H of CH_2(β)(Pro), 1 H of CH_2(γ)(Pro), CH(β)(Ile)); 1.65, 1.62 (2*s*, 2 Me(Aib)); 1.53–1.49, 1.16–1.10 (2*m*, CH_2(γ)(Ile)); 0.92 (*d*, *J* = 7.0, *Me*CH(β)(Ile)); 0.90 (*t*, *J* = 7.4, *Me*CH_2(γ)(Ile)). ^{13}C-NMR (150 MHz): 172.0 (*s*, CO(Aib)); 172.0 (*s*, CO(Pro)); 169.6 (*s*, CO(Ile)); 156.2 (*s*, CO(carbamate)); 136.4 (*s*, 1 arom. C); 131.9 (*d*, CH_2=*C*H); 128.5, 128.2, 128.0 (3*d*, 5 arom. CH); 118.5 (*t*, *C*H_2=CH); 66.9 (*t*, CH_2(carbamate)); 65.7 (*t*, CH_2O); 61.0 (*d*, CH(α)(Pro)); 59.7 (*d*, CH(α)(Ile)); 57.1 (*s*, C(α)(Aib)); 48.1 (*t*, CH_2(δ)(Pro)); 37.8 (*d*, CH(β)(Ile)); 27.8 (*t*, CH_2(β)(Pro)); 25.8 (*t*, CH_2(γ)(Pro)); 24.9 (*t*, CH_2(γ)(Ile)); 23.5, 23.1 (2*q*, 2 Me(Aib)); 15.4 (*q*, *Me*CH(β)(Ile)); 11.4 (*q*, *Me*CH_2(γ)(Ile)). ESI-MS (MeOH): 510 (100, [*M*+Na]$^+$). Anal. calc. for $C_{26}H_{37}N_3O_6$ (487.59): C 64.05, H 7.65, N 8.62; found: C 63.89, H 7.40, N 8.58.

Phenacyl N-*{2-[((S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenyl-1-oxopropyl)amino]-2-methyl-1-oxopropyl}*-L-*prolinate* (**13**). A soln. of **3a** (120 mg, 0.380 mmol) in CH_2Cl_2 (3 ml) was added to a soln. of Z-Phe-OH (120 mg, 0.401 mmol) in CH_2Cl_2 (3 ml) at 0°. The mixture was stirred at r.t. overnight, the solvent was evaporated, and the crude product was purified by CC (SiO_2, CH_2Cl_2/MeOH 80:1 → 60:1) to give **13** (213 mg, 94%). Colorless powder. M.p. 76.7–78.4°. IR: 3311*s*, 3062*m*, 3031*w*, 2983*m*, 2940*m*, 2880*w*, 1752v*s*, 1703v*s*, 1670v*s*, 1625v*s*, 1532v*s*, 1498*s*, 1469*s*, 1451*s*, 1412v*s*, 1377*s*, 1364*s*, 1341*m*, 1286*s*, 1232v*s*, 1166v*s*, 1097*w*, 1084*w*, 1052*m*, 1028*m*, 1001*w*, 977*m*, 913*w*, 848*w*, 752*s*, 698*s*. ^{1}H-NMR (600 MHz): 7.88–7.87, 7.61–7.59, 7.49–7.46, 7.37–7.20 (4*m*, 15 arom. H); 6.57 (*s*, NH(Aib)); 5.55, 5.18 (*AB*, *J* = 16.5, CH_2CO); 5.39 (*d*, *J* = 7.2, NH(Phe)); 5.10, 5.08 (*AB*, *J* = 12.0, CH_2(carbamate)); 4.68–4.66 (*m*, CH(α)(Pro)); 4.38–4.37 (*m*, CH(α)(Phe)); 3.66–3.59, 3.46–3.42 (2*m*, CH_2(δ)(Pro)); 3.11–3.08, 3.03–3.00 (2*m*, CH_2(Phe)); 2.32–2.26, 2.19–2.13 (2*m*, CH_2(β)(Pro)); 2.10–2.06, 1.92–1.84 (2*m*, CH_2(γ)(Pro)); 1.50, 1.42 (2*s*, 2 Me(Aib)). ^{13}C-NMR (150 MHz): 192.2 (*s*, CO(carbonyl)); 171.8 (*s*, CO(Aib), CO(Pro)); 169.1 (*s*, CO(Phe)); 155.9 (*s*, CO(carbamate)); 136.3, 136.2, 134.1 (3*s*, 3 arom. C); 134.0, 129.5, 128.9, 128.7, 128.6, 128.2, 128.0, 127.7, 127.1 (9*d*, 15 arom. CH); 67.0 (*t*, CH_2(carbamate)); 66.1 (*t*, *C*H_2CO); 60.8 (*d*, CH(α)(Pro)); 56.9 (*s*, C(α)(Aib)); 56.4 (*d*, CH(α)(Phe)); 48.1 (*t*, CH_2(δ)(Pro)); 38.7 (*t*, CH_2(Phe)); 27.8 (*t*, CH_2(β)(Pro)); 25.8 (*t*, CH_2(γ)(Pro)); 23.7, 23.4 (2*q*, 2 Me(Aib)). ESI-MS (MeOH): 622 (100, $[M+Na]^+$). Anal. calc. for $C_{34}H_{37}N_3O_7$ (599.67): C 68.10, H 6.22, N 7.01; found: C 68.13, H 6.27, N 6.90.

N-*{2-[((S)-2-{[(Benzyloxy)carbonyl]amino}-3-phenyl-1-oxopropyl)amino]-2-methyl-1-oxopropyl}*-L-*proline* (**14**). Zn-powder (274 mg, 4.190 mmol) was added to a soln. of **13** (50 mg, 0.083 mmol) in AcOH (100%, 2 ml) and the mixture was stirred at r.t. for 45 min. Additional Zn-powder (136 mg, 2.080 mmol) and AcOH (100%, 0.5 ml) were added and the mixture was stirred for further 45 min. The mixture was filtered, the residue washed with AcOH, and the filtrate was concentrated under reduced pressure. Prep. TLC (CH_2Cl_2/MeOH 10:1, 2 × dev.) yielded **14** (30 mg, 75%). Colorless powder. M.p. 94.4–96.3°. IR: 3305v*s*, 3063*s*, 3032*s*, 2984*s*, 2945*s*, 2879*m*, 1720v*s*, 1666v*s*, 1620v*s*, 1537v*s*, 1498v*s*, 1469*s*, 1454v*s*, 1419v*s*, 1383*s*, 1366*s*, 1341*s*, 1294*sh*, 1245v*s*, 1217*s*, 1178*s*, 1152*s*, 1084*w*, 1053*s*, 1028*m*, 912*w*, 744*s*, 699v*s*. ^{1}H-NMR (600 MHz): 7.37–7.21 (*m*, 10 arom. H); 6.58 (br. *s*, NH(Aib)); 5.43 (br. *s*, NH(Phe)); 5.12, 5.07 (*AB*, *J* = 12.1, CH_2(carbamate)); 4.57–4.54 (*m*, CH(α)(Pro)); 4.41 (*q*-like, *J* ≈ 7.4, CH(α)(Phe)); 3.39–3.35 (*m*, 1 H of CH_2(δ)(Pro)); 3.07–3.05 (*m*, CH_2(Phe)); 2.96–2.94 (*m*, 1 H of CH_2(δ)(Pro)); 2.09–1.95 (*m*, CH_2(β)(Pro)); 1.78–1.68 (*m*, CH_2(γ)(Pro)); 1.37 (*s*, 2 Me(Aib)); COOH could not be detected. ^{13}C-NMR (150 MHz): 173.0

(*s*, CO(Pro)); 172.7 (*s*, CO(Aib)); 170.4 (*s*, CO(Phe)); 156.3 (*s*, CO(carbamate)); 136.0, 135.9 (2*s*, 2 arom. C); 129.5, 128.8, 128.6, 128.4, 128.1, 127.2 (6*d*, 10 arom. CH); 67.4 (*t*, CH_2(carbamate)); 61.6 (*d*, CH(α)(Pro)); 56.9 (*s*, C(α)(Aib)); 56.1 (*d*, CH(α)(Phe)); 48.2 (*t*, CH_2(δ)(Pro)); 37.4 (*t*, CH_2(Phe)); 27.3 (*t*, CH_2(β)(Pro)); 25.9 (*t*, CH_2(γ)(Pro)); 24.8, 24.3 (2*q*, 2 Me(Aib)). ESI-MS (MeOH): 504 (100, $[M+Na]^+$). Anal. calc. for $C_{26}H_{31}N_3O_6 \cdot H_2O$ (499.56): C 62.51, H 6.66, N 8.41; found: C 62.80, H 6.47, N 8.27.

N-*{2-[(2(*S*),3(*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-methyl-1-oxopentyl)amino]-2-methyl-1-oxopropyl}*-L-*proline* (**15**). A soln. of $Pd(Ph_3P)_4$ (*ca*. 4 mg, *ca*. 0.004 mmol) and $PhSiH_3$ (70 µl, 0.567 mmol) in CH_2Cl_2 (1 ml) was added to a soln. of **12b** (70 mg, 0.144 mmol) in CH_2Cl_2 (1 ml) and the mixture was stirred at r.t. under Ar and exclusion of light for 50 min. The mixture was concentrated *i.v*. and purified by prep. TLC (CH_2Cl_2/MeOH 10:1, 3 × dev.) to give **15** (54 mg, 84%). Colorless powder. M.p. 101.5–103.2°. IR: 3426*s*, 3306v*s*, 3063*s*, 3035*s*, 2966v*s*, 2936*s*, 2878*s*, 1705v*s*, 1659v*s*, 1622v*s*, 1535v*s*, 1469*s*, 1454v*s*, 1416v*s*, 1384*s*, 1365*s*, 1342*s*, 1309*s*, 1288*s*, 1245v*s*, 1178*s*, 1128*m*, 1094*m*, 1042*s*, 1028*m*, 983*w*, 914*w*, 882*w*, 778*w*, 739*m*, 698*s*. ^{1}H-NMR (600 MHz): 7.34–7.31 (*m*, 5 arom. H); 7.10 (br. *s*, NH(Aib)); 5.69 (br. *s*, NH(Ile)); 5.11, 5.07 (*AB*, *J* = 11.8, CH_2(carbamate)); 4.55 (br. *s*, CH(α)(Pro)); 3.97 (br. *s*, CH(α)(Ile)); 3.49 (br. *s*, CH_2(δ)(Pro)); 2.08–1.83 (*m*, CH_2(β)(Pro), 1 H of CH_2(γ)(Pro), CH(β)(Ile)); 1.75–1.67 (*m*, 1 H of CH_2(γ)(Pro)); 1.53, 1.51 (2*s*, 2 Me(Aib)); *ca*. 1.50, 1.67–1.09 (2*m*, CH_2(γ)(Ile)); 0.92 (*d*, *J* = 7.1, *Me*CH(β)(Ile)); 0.88 (*t*, *J* = 7.1, *Me*CH_2(γ)(Ile)); COOH could not be detected. ^{13}C-NMR (150 MHz): 173.9, 173.0, 171.1 (3*s*, 3 CO); 156.6 (*s*, CO(carbamate)); 136.2 (*s*, 1 arom. C); 128.6, 128.3, 128.0 (3*d*, 5 arom. CH); 67.1 (*t*, CH_2(carbamate)); 61.7 (*d*, CH(α)(Pro)); 59.8 (*d*, CH(α)(Ile)); 57.0 (*s*, C(α)(Aib)); 48.2 (*t*, CH_2(δ)(Pro)); 36.8 (*d*, CH(β)(Ile)); 27.4 (*t*, CH_2(β)(Pro)); 25.9 (*t*, CH_2(γ)(Pro)); 24.8 (*t*, CH_2(γ)(Ile)); 24.5, 24.4 (2*q*, 2 Me(Aib)); 15.5 (*q*, *Me*CH(β)(Ile)); 11.2 (*q*, *Me*CH_2(γ)(Ile)). ESI-MS (MeOH): 486 (19, $[M+K]^+$), 470 (100, $[M+Na]^+$), 305 (57, $[M–Pro–CO]^+$). Anal. calc. for $C_{23}H_{33}N_3O_6 \cdot H_2O$ (465.54): C 59.34, H 7.58, N 9.03; found: C 59.46, H 7.41, N 8.69.

5. *Synthesis of Thioamides* **8a–c**. *(9*H*-Fluoren-9-yl)methyl* N-*(2-Methylpropanoyl)*-L-*prolinate* (**7a**). At 0°, DCC (1.227 g, 5.95 mmol) was added to a soln. of **6** (1.000 g, 5.40 mmol), 9*H*-fluoren-9-ylmethanol (1.166 g, 5.94 mmol) and PPY (42 mg, 0.283 mmol) in CH_2Cl_2 (50 ml). After stirring at r.t. for 4 h, the mixture was filtered, and the solvent was evaporated. CC (SiO_2, AcOEt/hexane: 4:6 → 6:4) yielded **7a** (1.786 g, 91%). Colorless powder. M.p. 104.4–107.3°. IR: 2971*m*, 2951*m*, 2932*m*, 2892*w*, 2873*m*, 1750v*s*, 1707*w*, 1629v*s*, 1536*w*, 1471*s*, 1446*s*, 1432v*s*, 1381*m*, 1329*s*, 1320*m*, 1270*m*, 1196*s*, 1167v*s*, 1088*m*, 1035*m*, 1024*w*, 1003*w*, 955*w*, 945*w*, 903*w*, 752*s*, 741*s*. ^{1}H-NMR (300 MHz, conformers

(86:14)): 7.78–7.73, 7.64–7.54, 7.42–7.27 (3*m*, 8 arom. H); 4.64–4.59 (*m*, CH(α)(Pro)); 4.53–4.43 (*m*, CH_2(Fm)); 4.21 (*t*, *J* = 6.3, CH(Fm)); 3.52–3.46 (*m*, CH_2(δ)(Pro)); 2.60 (*sept.*, *J* = 6.8, Me_2CH); 2.11–2.01, 1.91–1.65 (2*m*, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.10, 1.06 (2*d*, *J* = 6.8, 2 Me). ^{13}C-NMR (75 MHz): 175.9, 172.4 (2*s*, 2 CO); 144.1, 143.7, 141.4, 141.4 (4*s*, 4 arom. C); 127.8, 127.2, 125.1, 125.0, 119.9 (5*d*, 8 arom. CH); 66.2 (*t*, CH_2(Fm)); 58.8 (*d*, CH(α)(Pro)); 47.1 (*d*, CH(Fm)); 46.7 (*t*, CH_2(δ)(Pro)); 32.3 (*d*, Me_2CH); 29.0, 24.7 (2*t*, CH_2(β)(Pro), CH_2(γ)(Pro)); 18.8, 18.7 (2*q*, 2 Me). ESI-MS (MeOH): 414 (19, $[M+H+H_2O+MeOH]^+$), 386 (100, $[M+Na]^+$).

2-[(4-Nitrophenyl)sulfonyl]ethyl N-*(2-Methylpropanoyl)*-L-*prolinate* (**7b**). A soln. of 2-(4-nitrophenylsulfonyl)ethanol (433 mg, 1.873 mmol), EDCI (474 mg, 2.473 mmol), DMAP (24 mg, 0.196 mmol) and **6** (381 mg, 2.057 mmol) in CH_2Cl_2 (30 ml) was stirred under N_2 at r.t. for 1 d. Aq. AcOH (5%, *ca.* 40 ml) was added, and the mixture was extracted with CH_2Cl_2. The combined org. layers were dried (Na_2SO_4) and concentrated *i.v.* CC (SiO_2, CH_2Cl_2/MeOH 50:1) yielded **7b** (683 mg, 92%). Colorless powder. M.p. 89.8–91.9°. IR: 3463*w*, 3119*w*, 2975*s*, 2931*m*, 2879*w*, 1740v*s*, 1642v*s*, 1608*m*, 1530v*s*, 1467*m*, 1428v*s*, 1391*m*, 1351v*s*, 1330v*s*, 1312v*s*, 1302v*s*, 1270*w*, 1248*m*, 1210*m*, 1179v*s*, 1146v*s*, 1104*m*, 1089*s*, 1046*w*, 1010*m*, 994*w*, 919*w*, 857*m*, 840*w*, 805*w*, 757*s*, 739*s*, 703*s*. ^{1}H-NMR (300 MHz, conformers (96:4)): 8.44–8.40, 8.20–8.15 (2*m*, 4 arom. H); 4.51–4.46 (*m*, CH_2CH_2O); 4.16–4.12 (*m*, CH(α)(Pro)); 3.67–3.49 (*m*, CH_2CH_2O, CH_2(δ)(Pro)); 2.64 (*sept.*, *J* = 6.8, Me_2CH); 2.11–1.86 (*m*, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.12, 1.10 (2*d*, *J* = 6.8, 2 Me). ^{13}C-NMR (75 MHz): 176.0 (*s*, CO(amide)); 171.8 (*s*, CO(ester)); 151.0, 144.8 (2*s*, 2 arom. C); 129.9, 124.6 (2*d*, 4 arom. CH); 58.5 (*d*, CH(α)(Pro)); 57.5 (*t*, CH_2CH_2O); 55.1 (*t*, CH_2CH_2O); 46.8 (*t*, CH(δ)(Pro)); 32.2 (*d*, Me_2CH); 28.8, 25.0 (2*t*, CH_2(β)(Pro), CH_2(γ)(Pro)); 18.8, 18.7 (2*q*, 2 Me). ESI-MS (MeOH, NaI): 421 (100, $[M+Na]^+$).

2-[(4-Nitrophenyl)sulfanyl]ethyl N-*(2-Methylpropanoyl)*-L-*prolinate* (**7c**). At 0°, DCC (513 mg, 2.486 mmol) was added to a soln. of **6** (420 mg, 2.268 mmol), 2-(4-nitrophenylsulfanyl)ethanol (470 mg, 2.359 mmol) and PPY (38 mg, 0.256 mmol) in CH_2Cl_2 (20 ml). After stirring at r.t. for 5.5 h, the mixture was filtered, and the solvent was evaporated. CC (SiO_2, AcOEt/hexane: 6:4) yielded **7c** (807 mg, 97%). Yellow oil. IR (film): 3469*w*, 3327*w*, 3100*m*, 3069*w*, 2972v*s*, 2933v*s*, 2876*s*, 1745v*s*, 1643v*s*, 1594v*s*, 1578v*s*, 1512v*s*, 1471v*s*, 1427v*s*, 1381v*s*, 1338v*s*, 1322*sh*, 1274v*s*, 1243*s*, 1171v*s*, 1090v*s*, 1046*s*, 1009*s*, 966*m*, 953*m*, 916*w*, 886*w*, 853v*s*, 742v*s*. ^{1}H-NMR (600 MHz, conformers (92:8)): 8.18–8.13, 7.44–7.40 (2*m*, 4 arom. H); 4.45–4.43 (*m*, CH(α)(Pro)); 4.39–4.30 (*m*, CH_2CH_2O); 3.71–3.67, 3.60–3.56 (2*m*, CH_2(δ)(Pro)); 3.31–3.27 (*m*, CH_2CH_2O); 2.69 (*sept.*, *J* = 6.8,

Me_2CH); 2.21–2.16, 2.11–2.07, 2.02–1.93 (3*m*, $CH_2(\beta)$(Pro), $CH_2(\gamma)$(Pro)); 1.16, 1.13 (2*d*, *J* = 6.8, 2 Me). ^{13}C-NMR (150 MHz): 176.0 (*s*, CO(amide)); 172.3 (*s*, CO(ester)); 146.0, 145.4 (2*s*, 2 arom. C); 126.7, 124.1 (2*d*, 4 arom. CH); 62.5 (*t*, CH_2CH_2O); 58.8 (*d*, $CH(\alpha)$(Pro)); 46.8 (*t*, $CH_2(\delta)$(Pro)); 32.3 (*d*, Me_2CH); 30.3 (*t*, CH_2CH_2O); 29.1 (*t*, $CH_2(\beta)$(Pro)); 25.0 (*t*, $CH_2(\gamma)$(Pro)); 18.9, 18.7 (2*q*, 2 Me). ESI-MS (CH_2Cl_2,MeOH, NaI): 389 (100, $[M+Na]^+$).

*(9*H*-Fluoren-9-yl)methyl* N*-(2-Methylpropanthioyl)*-L*-prolinate* (**8a**). A suspension of *Lawesson* reagent (dried *i.v.*, 307 mg, 0.759 mmol) and **7a** (499 mg, 1.373 mmol) in toluene (15 ml) was heated at 95° (oilbath) for 1 h. After cooling to r.t., the mixture was filtered, and the solvent was evaporated. CC (SiO_2, AcOEt/hexane 2:8) yielded **8a** (369 mg, 71%). Colorless powder. M.p. 174.1–176.3°. IR: 2974*m*, 2964*m*, 2927*m*, 2873*w*, 1743v*s*, 1595*w*, 1554*w*, 1503*w*, 1461*s*, 1440v*s*, 1383*s*, 1359*m*, 1345*s*, 1336*s*, 1300*w*, 1270*s*, 1255*m*, 1229*m*, 1194v*s*, 1171v*s*, 1156v*s*, 1123*s*, 1020*m*, 1006*s*, 975*m*, 939*m*, 761*s*, 742v*s*, 716*w*. ^{1}H-NMR (300 MHz, conformers (88:12)): 7.78–7.58, 7.42–7.27 (2*m*, 8 arom. H); 5.10–5.06 (*m*, $CH(\alpha)$(Pro)); 4.73 (*dd*, *J* = 10.8, 5.6, 1 H of CH_2(Fm)); 4.42 (*dd*, *J* = 10.8, 6.4, 1 H of CH_2(Fm)); 4.24 (*t*-like, $J \approx 6.0$, CH(Fm)); 3.68–3.63 (*m*, $CH_2(\delta)$(Pro)); 2.94 (*sept.*, *J* = 6.6, Me_2CH); 2.17–2.07, 1.96–1.73 (2*m*, $CH_2(\beta)$(Pro), $CH_2(\gamma)$(Pro)); 1.21, 1.11 (2*d*, *J* = 6.5, 2 Me)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 209.7 (*s*, CS); 170.5 (*s*, CO); 144.0, 143.6, 141.4, 141.4 (4*s*, 4 arom. C); 127.8, 127.2, 125.2, 125.0, 119.9 (5*d*, 8 arom. H); 66.2 (*t*, CH_2(Fm)); 65.3 (*d*, $CH(\alpha)$(Pro)); 50.1 (*t*, $CH_2(\delta)$(Pro)); 47.1 (*d*, CH(Fm)); 38.7 (*d*, Me_2CH); 28.7, 24.5 (2*t*, $CH_2(\beta)$(Pro), $CH_2(\gamma)$(Pro)); 22.6, 22.4 (2*q*, 2 Me). ESI-MS (MeOH): 402 (100, $[M+Na]^+$).

2-[(4-Nitrophenyl)sulfonyl]ethyl N*-(2-Methylpropanthioyl)*-L*-prolinate* (**8 b**). A suspension of *Lawesson* reagent (dried *i.v.*, 374 mg, 0.925 mmol) and **7b** (662 mg, 1.662 mmol) in toluene (20 ml) was heated at 90° (oilbath) for 1 h. After cooling to r.t., the mixture was filtered, and the solvent was evaporated. CC (SiO_2, CH_2Cl_2/MeOH 70:1) yielded **8b** (509 mg, 74%). Yellow powder. M.p. 174.1–176.3°. IR: 3470*w*, 3109*w*, 3062*w*, 3033*w*, 2966*s*, 2924*s*, 2887*m*, 2864*w*, 1742v*s*, 1606*m*, 1524v*s*, 1475v*s*, 1458v*s*, 1402*m*, 1377*s*, 1350v*s*, 1327v*s*, 1303v*s*, 1264*s*, 1253*s*, 1226*s*, 1196v*s*, 1174v*s*, 1152v*s*, 1124*s*, 1106*m*, 1086*s*, 1070*s*, 1013*s*, 999*m*, 977*m*, 921*w*, 881*w*, 851*s*, 828*w*, 789*w*, 756v*s*, 738v*s*, 702v*s*. ^{1}H-NMR (300 MHz, conformers (95:5)): 8.47–8.41, 8.20–8.14 (2*m*, 4 arom. H); 4.77–4.73 (*m*, $CH(\alpha)$(Pro)); 4.61–4.44 (*m*, CH_2CH_2O); 4.13–3.85, 3.78–3.69 (2*m*, $CH_2(\delta)$(Pro)); 3.63–3.46 (*m*, CH_2CH_2O); 3.02 (*sept.*, *J* = 6.6, Me_2CH); 2.24–1.98 (*m*, $CH_2(\beta)$(Pro), $CH_2(\gamma)$(Pro)); 1.21, 1.21 (2*d*, *J* = 6.5, 2 Me). ^{13}C-NMR (75 MHz): 209.9 (*s*, CS); 169.8 (*s*, CO); 151.0, 144.8 (2*s*, 2 arom. C); 129.9, 124.6 (2*d*, 4 arom. CH); 65.0 (*d*, $CH(\alpha)$(Pro)); 57.6 (*t*, CH_2CH_2O); 55.1 (*t*,

CH_2CH_2O); 50.3 (*t*, CH_2(*δ*)(Pro)); 38.7 (*d*, Me_2CH); 28.5, 24.8 (2*t*, CH_2(*β*)(Pro), CH_2(*γ*)(Pro)); 22.7, 22.4 (2*q*, 2 Me). ESI-MS (MeOH, NaI): 437 (100, $[M+Na]^+$), 206 (25).

2-[(4-Nitrophenyl)sulfanyl]ethyl N-*(2-Methylpropanthioyl)*-L-*prolinate* (**8c**). A suspension of *Lawesson* reagent (dried *i.v.*, 473 mg, 1.170 mmol) and **7c** (773 mg, 2.110 mmol) in toluene (20 ml) was heated at 90° (oilbath) for 1 h. After cooling to r.t., the mixture was filtered, and the solvent was evaporated. CC (SiO_2, CH_2Cl_2/MeOH 300:1 → 100:1) yielded **8c** (599 mg, 74%). Yellow oil. IR (film): 3098*w*, 3067*w*, 2971v*s*, 2929*s*, 2878*s*, 1743v*s*, 1594v*s*, 1578v*s*, 1513v*s*, 1479v*s*, 1462v*s*, 1441v*s*, 1381v*s*, 1338v*s*, 1300*s*, 1268v*s*, 1257v*s*, 1227v*s*, 1188v*s*, 1166v*s*, 1125*s*, 1090v*s*, 1049*m*, 1016v*s*, 970*s*, 922*w*, 912*w*, 877*w*, 853v*s*, 841*s*, 779*w*, 742v*s*. ^{1}H-NMR (300 MHz, conformers (93:7)): 8.16–8.13, 7.45–7.41 (2*m*, 4 arom. H); 5.05–5.01 (*m*, CH(*α*)(Pro)); 4.41–4.30 (*m*, CH_2CH_2O); 3.91–3.89, 3.82–3.76 (2*m*, CH_2(*δ*)(Pro)); 3.31 (*t*, *J* = 7.1, CH_2CH_2O); 3.06 (*sept.*, *J* = 6.6, Me_2CH); 2.29–2.07 (*m*, CH_2(*β*)(Pro), CH_2(*γ*)(Pro)); 1.25, 1.25 (2*d*, *J* = 6.6, 2 Me). ^{13}C-NMR (75 MHz): 210.1 (*s*, CS); 170.5 (*s*, CO); 146.1, 145.6 (2*s*, 2 arom. C); 126.8, 124.3 (2*d*, 4 arom. CH); 65.4 (*d*, CH(*α*)(Pro)); 62.8 (*t*, CH_2CH_2O); 50.5 (*t*, CH_2(*δ*)(Pro)); 38.9 (*d*, Me_2CH); 30.3 (*t*, CH_2CH_2O); 29.0, 25.0 (2*t*, CH_2(*β*)(Pro), CH_2(*γ*)(Pro)); 22.9, 22.5 (2*q*, 2 Me). ESI-MS (MeOH, NaI): 405 (100, $[M+Na]^+$).

6. *X-Ray Crystal-Structure Determination of* **11a** (*Table* and *Fig.*)[4]). A crystal of $C_{24}H_{26}N_2O_4S$, obtained from $CDCl_3$/Et_2O, was used for a low-temp. X-ray crystal-structure determination. All measurements were made on a *Nonius KappaCCD* area-detector diffractometer [33] using graphite-monochromated MoK_α radiation (*λ* 0.71073 Å) and an *Oxford Cryosystems Cryostream 700* cooler. The data collection and refinement parameters are given in the *Table*, and a view of the molecule is shown in the *Figure*. Data reduction was performed with *HKL Denzo* and *Scalepack* [34]. The intensities were corrected for *Lorentz* and polarization effects, and an absorption correction based on the multi-scan method [35] was applied. Equivalent reflections, other than *Friedel* pairs, were merged. The structure was solved by direct methods using SIR92 [36], which revealed the positions of all non-H-atoms. The non-H-atoms were refined anisotropically. The amide H-atom was placed in the position indicated by a difference electron density map and its position was allowed to refine together with an isotropic displacement parameter. All remaining H-atoms were placed in

[4]) CCDC 602724 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the *Cambridge Crystallographic Data Center via* http://www.ccdc.cam.ac.uk/data_request/cif.

geometrically calculated positions and refined using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to $1.2U_{eq}$ of its parent C-atom ($1.5U_{eq}$ for the Me groups). Refinement of the structure was carried out on F^2 using full-matrix least-squares procedures, which minimised the function $\Sigma w(F_o^2 - F_c^2)^2$. A correction for secondary extinction was applied. Refinement of the absolute structure parameter [37] yielded a value of -0.08(6), which confidently confirms that the refined coordinates, represent the true enantiomorph. Neutral atom scattering factors for non-H atoms were taken from [38a], and the scattering factors for H-atoms were taken from [39]. Anomalous dispersion effects were included in F_c [40]; the values for f' and f'' were those of [38b]. The values of the mass attenuation coefficients are those of [38c]. All calculations were performed using the SHELXL97 [41] program.

Table. *Crystallographic Data for Compound* **11a**

Crystallised from	$CDCl_3/Et_2O$
Empirical formula	$C_{24}H_{26}N_2O_4S$
Formula weight [g mol^{-1}]	438.54
Crystal color, habit	colorless, needle
Crystal dimensions [mm]	0.08 × 0.10 × 0.30
Temp. [K]	160(1)
Crystal system	monoclinic
Space group	$P2_1$
Z	2
Reflections for cell determination	30655
2θ range for cell determination [°]	4 – 55
Unit cell parameters a [Å]	9.2417(3)
b [Å]	11.1230(4)
c [Å]	11.4726(4)
β [°]	110.418(2)
V [Å^3]	1105.24(7)
D_x [g cm^{-3}]	1.318
μ(MoK_α) [mm^{-1}]	0.180
Scan type	ϕ and ω
$2\theta_{(max)}$ [°]	55
Transmission factors (min; max)	0.799; 0.994
Total reflections measured	24199
Symmetry independent reflections	5044
Reflections with $I > 2\sigma(I)$	4212
Reflections used in refinement	5044
Parameters refined; restraints	287; 1
Final $R(F)$ [$I > 2\sigma(I)$ reflections]	0.0391
$wR(F^2)$ (all data)	0.0862
Weights: $w = [\sigma^2(F_o^2) + (0.0369P)^2 + 0.2109P]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$	
Goodness of fit	1.030
Final Δ_{max}/σ	0.001
$\Delta\rho$ (max; min) [e Å^{-3}]	0.19; -0.22

References

[1] W. F. DeGrado, *Adv. Prot. Chem.* **1988**, *39*, 51.

[2] J. Rizo, L. M. Gierasch, *Annu. Rev. Biochem.* **1992**, *61*, 387.

[3] V. J. Hruby, F. Al-Obeidi, W. Kazmierski, *Biochem. J.* **1990**, *268*, 249.

[4] M. Mutter, *Angew. Chem., Int. Ed.* **1985**, *24*, 639.

[5] H. Brückner, T. Kripp, M. Kiess, in 'Peptides 1990', Eds. E. Giralt, D. Andreu, ESCOM, Leiden, 1991, p. 387; C. Auvin-Guette, S. Rebuffat, Y. Prigent, B. Bodo, *J. Am. Chem. Soc.* **1992**, *114*, 2170.

[6] H. Heimgartner, *Angew. Chem., Int. Ed.* **1991**, *30*, 238.

[7] J. M. Humphrey, A. R. Chamberlin, *Chem. Rev.* **1997**, *97*, 2243.

[8] P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1986**, *69*, 1153; **1987**, *70*, 354.

[9] N. Pradeille, A. Linden, K. Möhle, O. Zerbe, H. Heimgartner, *Chem. Biodiv.* **2005**, *2*, 1127.

[10] S. A. Stoykova, Ph.D. thesis, Universität Zürich, 2005.

[11] R. T. N. Luykx, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2003**, *86*, 4093.

[12] N. Pradeille, H. Heimgartner, *J. Pept. Sci.* **2003**, *9*, 827.

[13] S. Stamm, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2003**, *86*, 1371.

[14] K. A. Brun, Ph.D. thesis, Universität Zürich, 2002.

[15] J. Lehmann, A. Linden, H. Heimgartner, *Tetrahedron* **1998**, *54*, 8721.

[16] C. B. Bucher, H. Heimgartner, *Helv. Chim. Acta* **1996**, *79*, 1903.

[17] W. Altherr, Ph.D. thesis, Universität Zürich, 1994.

[18] P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1990**, *73*, 13.

[19] S. Stamm, H. Heimgartner, *Eur. J. Org. Chem.* **2004**, 3820.

[20] S. Stamm, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2006**, *89*, 1.

[21] L. Whitmore, B. A. Wallace, *Nucleic Acid Research* **2004**, *32*, D593–D594.

[22] R. T. N. Luykx, C. B. Bucher, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **1996**, *79*, 527.

[23] S. Stamm, H. Heimgartner, in preparation.

[24] H. Kessler, R. Siegmeier, *Tetrahedron Lett.* **1983**, *24*, 281.

[25] M. A. Bednarek, M. Bodanszky, *Int. J. Pept. Protein Res.* **1983**, *21*, 196.

[26] D. A. Alonso, C. Nájera, M. Varea, *Synthesis* **2003**, 277.

[27] P. Wipf, Ph.D. thesis, Universität Zürich, 1987.

[28] J. B. Hendrickson, C. Kandall, *Tetrahedron Lett.* **1970**, *11*, 343.

[29] M. Dessolin, M.-G. Guillerez, N. Thieriet, F. Guibé, A. Loffet, *Tetrahedron Lett*. **1995**, *36*, 5741.

[30] J. Bernstein, R.E. Davis, L. Shimoni, N.-L. Chang, *Angew. Chem. Int. Ed. Engl*. **1995**, *34*, 1555.

[31] C. K. Johnson, *ORTEPII*, Report ORNL-5138, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1976.

[32] M. Namikoshi, B. Kundu, K. L. Rinehart, *J. Org. Chem*. **1991**, *56*, 5464.

[33] R. Hooft, *KappaCCD Collect Software*, Nonius BV, Delft, The Netherlands, 1999.

[34] Z. Otwinowski, W. Minor, in *Methods in Enzymology*, Vol. 276, *Macromolecular Crystallography*, Part A, Eds. C. W. Carter Jr., R. M. Sweet, Academic Press, New York, 1997, p. 307.

[35] R. H. Blessing, *Acta Crystallogr., Sect A* **1995**, *51*, 33.

[36] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori, M. Camalli, *SIR92*, *J. Appl. Crystallogr*. **1994**, *27*, 435.

[37] a) H. D. Flack, G. Bernardinelli, *Acta Crystallogr., Sect A* **1999**, *55*, 908; b) H. D. Flack, G. Bernardinelli, *J. Appl. Crystallogr.,* **2000**, *33*, 1143.

[38] a) E. N. Maslen, A. G. Fox, M. A. O'Keefe, in 'International Tables for Crystallography', Ed. A. J. C. Wilson, Kluwer Academic Publishers, Dordrecht, 1992, Vol. C, Table 6.1.1.1, p. 477; b) D. C. Creagh, W. J. McAuley, *ibid*. Table 4.2.6.8, p. 219; c) D. C. Creagh, J. H. Hubbell, *ibid*. Table 4.2.4.3, p. 200.

[39] R. F. Stewart, E. R. Davidson, W. T. Simpson, *J. Chem. Phys*. **1965**, *42*, 3175.

[40] J. A. Ibers, W. C. Hamilton, *Acta Crystallogr*. **1964**, *17*, 781.

[41] G. M. Sheldrick, *SHELXL97, Program for the Refinement of Crystal Structures*, University of Göttingen, Germany, 1997.

7. Introduction of the Aib-Pro Unit into Peptides by means of the 'Azirine/ Oxazolone Method' on Solid Phase[1]

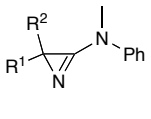
A method for the direct introduction of Aib-Pro into peptides on solid phase was developed. The Aib-Pro unit was introduced by means of the 'azirine/oxazolone method' using allyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate as the synthon. After the reaction of the resin-bound peptide acid with allyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate, the allyl protecting group of the resulting extended peptide could be removed by a mild Pd^0-promoted procedure. Cleavage of the peptide from the resin was performed with UV-light at 352 nm and yielded C-terminal protected peptides. The method found a successful application in the syntheses of different Aib-Pro containing peptaibol segments. Furthermore, a protected derivative of the peptide antibiotic *Trichovirin I 1B* was prepared by segment condensation.

1. Introduction

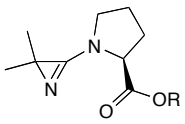
2*H*-Azirin-3-amines are highly-strained systems with versatile reactivity.[1] One very interesting and useful reaction is their application in peptide synthesis. In the 'azirine/oxazolone method', 2*H*-azirin-3-amines such as **1** or **2**, are used as synthons for the introduction of sterically demanding α,α-disubstituted α-amino acids into peptides.[1–3] Thus, the reaction of 2*H*-azirin-3-amines, e.g., the α-aminoisobutyric acid (Aib) synthon **1a**, with amino or peptide acids leads to peptide amides, the terminal amide bonds of which can be hydrolyzed selectively to give extended peptide acids. In solution-phase chemistry, the 'azirine/oxazolone method' has proven to be successful for the introduction of a variety of sterically demanding α,α-disubstituted α-amino acids into oligopeptides,[4–13] endothiopeptides,[14–16] cyclic peptides,[17–18] and cyclic depsipeptides.[19–23]

[1] S. Stamm, H. Heimgartner, *Tetrahedron*, submitted.

Recently, we adapted the 'azirine/oxazolone method' to solid-phase conditions, in order to additionally benefit from their advantages.[24] In this method, the growing peptide was attached through a carbamate linker to a [4-(hydroxymethyl)phenyl]acetamidomethyl (PAM) polystyrene resin (**3**) (Scheme 1). After deprotection of ^{t}Bu ester **4**, resin **5** was treated with a solution of **1a**. It is worth mentioning that unconsumed **1a** could easily be recovered and re-used. The terminal amide **6** was selectively hydrolyzed with 3M HCl in THF/H_2O to provide peptide acid resin **7**. Further extension of the peptide chain could be achieved either with a ^{t}Bu protected amino acid and a coupling reagent or with **1a**. Cleavage of the peptide from the resin was achieved with HBr (33%) in acetic acid, and yielded the tripeptide **8**. In a recent paper, we showed that the method is not restricted to the Aib synthon **1a**, and that it was successfully extended to the 1-aminocyclopentane-1-carboxylic acid synthon **1b**, the 4-amino-3,4,5,6-tetrahydro-2*H*-pyran-4-carboxylic acid synthon **1 c** , and the α-methylphenylalanine synthon **1d**.[25]



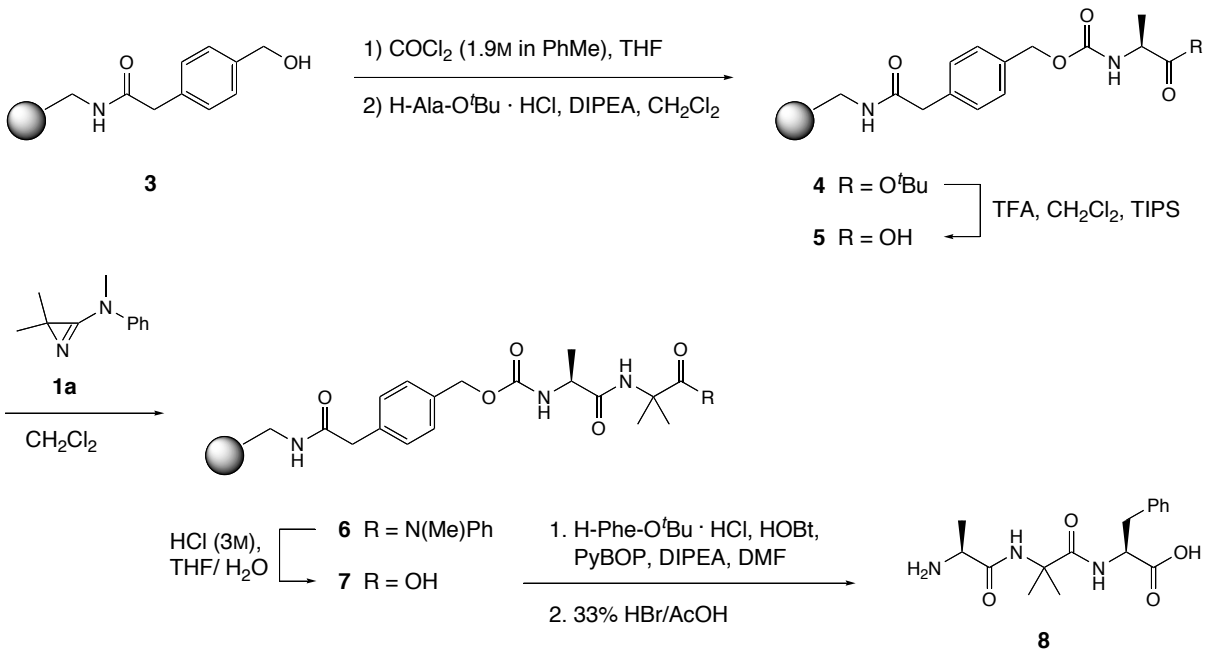
1a $R^1 = R^2 = Me$
1b $R^1 - R^2 = -(CH_2)_4-$
1c $R^1 - R^2 = -(CH_2)_2O(CH_2)_2-$
1d $R^1 = Me$, $R^2 = PhCH_2$



2a R = Me
2b R = CH_2CHCH_2
2c R = $PhCOCH_2$

Peptaibols are linear, amphiphilic oligopeptides from fungal sources with a high proportion of α,α-disubstituted α-amino acids, primarily, Aib.[26–27] Peptaibols show antibiotic properties due to self-association in lipid membranes forming ion channels.[28] Several peptaibols, or segments thereof, were synthesized by means of the 'azirine/oxazolone method'.[4,8–13] The 2*H*-azirine-3-amine **2a** played an important role in these syntheses, since **2a** allowed the direct introduction of the frequently present, but relatively acid labile, Aib-Pro unit.[9] Unexpectedly, the use of **2a** on solid phase was not successful (vide infra).

Herein we report a method for the introduction of the Aib-Pro unit into peptides on solid phase, using a photolabile linker and a new Aib-Pro synthon (**2b**), which was especially developed for this purpose.[29]



Scheme 1

2. Results and Discussion

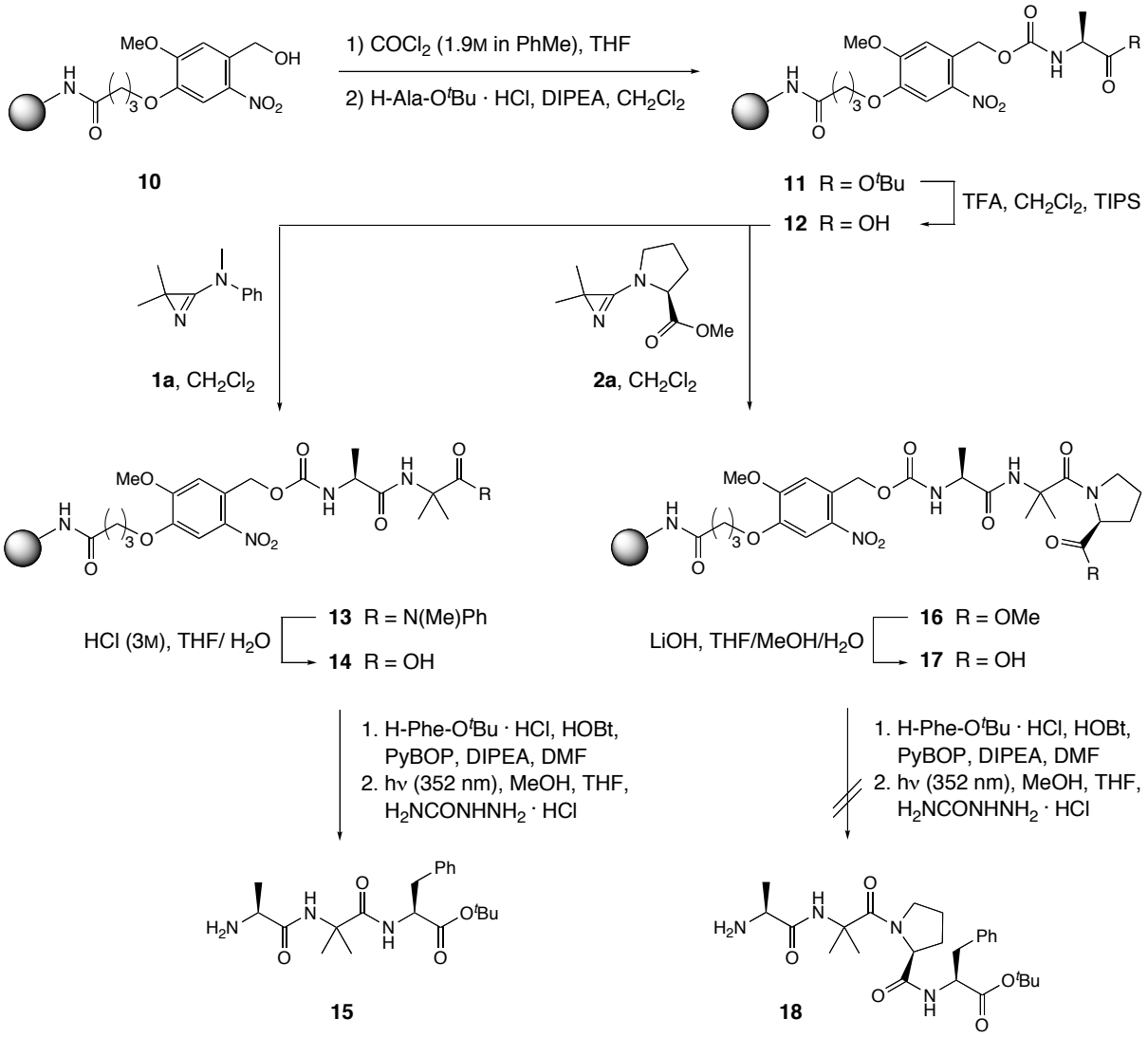
The most obvious approach to the introduction of the Aib-Pro unit was the use of synthon **2a** in analogy to the method outlined in Scheme 1. In doing so, resin **5** was treated with a solution of **2a**, and the resulting resin-bound tripeptide methyl ester was saponified with LiOH in a mixture of THF, MeOH, and H_2O. After coupling H-Val-O^{t}Bu and using PyBOP as the coupling reagent, the peptide was cleaved from the resin with HBr (33%) in acetic acid to give H-Ala-Aib-Pro-Val-OH (**9**) in 20% yield. In order to guarantee proper swelling of the resin during the saponification, the experiment was repeated with a Tentagel resin. However, the yield (21%) was still conspicuously lower, in comparison with the 37–50% obtained when introducing the synthons **1a–d**. The problem was recognized, when attempting to prepare the model peptide H-Ala-Aib-Pro-Leu-Aib-Val-OH (on Tentagel resin) and the peptaibol segment (A8–A14 of *Trichovirin Ia*) H-Val-Aib-Gly-Aib-Aib-Pro-Leu-OH (on polystyrene resin), whose syntheses failed completely. Only peptide fragments, which are caused by Aib-Pro fissions, were detected. The analysis of the fragments revealed that the cleavage of the Aib-Pro amide bond occurred during the HBr-promoted cleavage of the peptide from the resin, and not during the hydrolysis of the terminal amide, which was necessary after the incorporation of **1a**.

Thus, a linker was required which can be cleaved under milder conditions, but is still stable in TFA (50%) and HCl (3M). Some years ago, Kunz introduced a Pd^0-labile allyl linker for the synthesis of peptide acids.[30] Therefore, we synthesized 2-{[(*Z*)-4-(triisopropylsilyloxy)but-2-enyl]oxy}ethanoic acid[31] and attached its carboxy group via an alanine spacer to an aminomethyl polystyrene resin.[32] Additionally, (*E*)-7-hydroxyhept-5-enoic acid was prepared,[33] and its carboxy group was attached to an aminomethyl polystyrene resin. In both cases, the N-terminus of the first amino acid was immobilized through a carbamate group to the resin (in the first case after removing the TIPS-protecting group with TFA), and the resin bound peptides **8** and **9** were synthesized analogously to the 'PAM/HBr-strategy'. Cleavage from the resin was performed with $Pd(Ph_3P)_4$ and $PhSiH_3$ in $DMSO/CH_2Cl_2$, but the desired peptides could only be obtained in low yield and after a painstaking purification of the Pd-containing crude product.

In 1995, Holmes introduced a photolabile nitroveratryl linker and immobilized peptides by coupling their C-terminus to the resin.[34–36] This photolinker is among the most effective ever described – it is stable to various chemical reactions, but can easily be cleaved with UV-light with $\lambda = 365$ nm, a wavelength that does not affect aromatic amino acids such as Trp or Tyr. The corresponding photocleavable resin **10** (Scheme 2) is commercially available from Novabiochem.

With the aim of testing the stability under acidic conditions, *N*-(4,5-dimethoxy-2-nitrobenzyloxy)carbonyl-L-alanine *tert*-butyl ester (NVOC-Ala-O^{t}Bu) was prepared by reaction of 4,5-dimethoxy-2-nitrobenzyl alcohol and $COCl_2$ in THF, and subsequently with H-Ala-O^{t}Bu · HCl under Schotten-Baumann conditions to give NVOC-Ala-O^{t}Bu, which was then subjected to acidic conditions. Apart from ^{t}Bu hydrolysis, neither in TFA (50%), nor in HCl (3M) was cleavage or decomposition of the linker observed. Hence, the chloroformate of the photocleavable resin **10** was reacted with H-Ala-O^{t}Bu. Deprotection of the ^{t}Bu ester **11** with TFA afforded resin **12**, which was treated with a solution of **1a**. Selective hydrolysis of the terminal amide of resin **13** with 3M HCl afforded peptide-acid resin **14**, which was coupled to H-Phe-O^{t}Bu. Cleavage of the peptide from the resin was performed with UV-light (16 × 8 W, $\lambda_{max} = 352$ nm) in $MeCN/H_2O$, but the initial yields were disappointing. Most probably, the remaining nitrosoaldehyde functionalized resin captured the peptide (**15**) via its amino group.[37] This assumption was supported by ^{1}H-NMR experiments: the formyl signal of methyl 4-[(4-formyl-2-methoxy-2-nitrophenyl)oxy]butanoate disappeared within 20 min on reaction with isopropylamine, and a new signal at 8.65 ppm, typical for imines, appeared. To overcome the peptide-capture, the cleavage from the resin was performed in a solution of

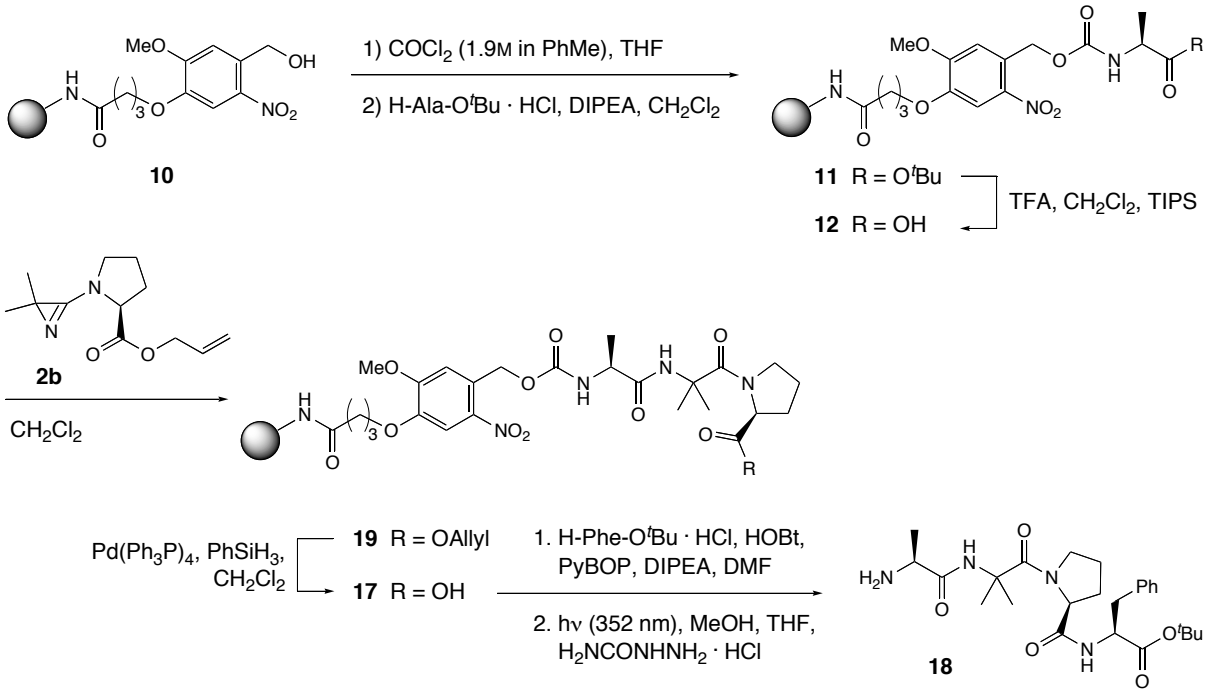
semicarbazide hydrochloride in THF/MeOH.[37] Although **15** was now obtained in much better yield (ca. 30–40%), its purity was unsatisfactory. Therefore, the general procedures had to be slightly modified again. The reaction time for the ^{t}Bu ester hydrolysis was extended to 60 min to avoid the previously detected H-Ala-Phe-O^{t}Bu side product. Moreover, the synthesis was carried out on a polystyrene instead of a Tentagel resin. The use of Tentagel resin suffered from (poly)ethylene glycol loss. Finally, model peptide **15** was prepared in high purity and in 33% yield (after prep. HPLC, based on resin loading).



Scheme 2

After having established the protocol for the use of Aib synthon **1a**, the preparation of Aib-Pro containing peptides was attempted. But when dipeptide synthon **2a** was used to incorporate the Aib-Pro unit, the experiment failed again (Scheme 2). During the saponification of resin-bound methyl ester **16** to give **17**, we observed a considerable darkening of the resin. Hence, quenching prevented the subsequent photo-induced cleavage of the peptide from the resin.

This issue was addressed by the synthesis of the new Aib-Pro synthons allyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2b**) and phenacyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2c**), which contain easily removable carboxy-protecting groups.[29] Resin **12** was reacted with a solution of **2c**, and the resulting phenacyl ester resin was treated with tetrabutylammonium fluoride. Again, considerable darkening of the resin was observed. On the other hand, the allyl protecting group of resin **19**, obtained from the reaction of resin **12** and **2b**, was smoothly removed with $Pd(Ph_3P)_4$ and $PhSiH_3$[38] in CH_2Cl_2 to give resin **17** (Scheme 3). The latter was then coupled with H-Phe-O^{t}Bu and PyBOP as the coupling reagent affording the corresponding resin bound tetrapeptide. Cleavage from the resin was achieved with UV-light (16 × 8 W, λ_{max} = 352 nm) in a solution of semicarbazide hydrochloride in THF/MeOH and gave H-Ala-Aib-Pro-Phe-O^{t}Bu (**18**) in high purity and in 35% yield (after prep. HPLC, based on resin loading).



Scheme 3

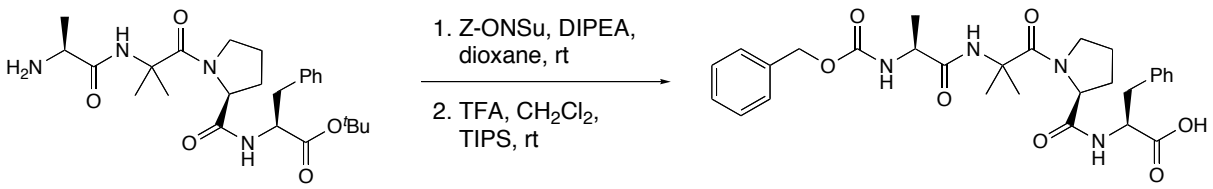
To evaluate the described protocol for the introduction of the Aib-Pro motif into peptides, the preparation of some peptaibol segments was attempted. The heptapeptide H-Val-Aib-Gly-Aib-Aib-Pro-Leu-O^{t}Bu (**20**, A8–A14 of *Trichovirin Ia*; Table 1), whose synthesis failed with the 'PAM/HBr-strategy' (see above), was prepared in 42% yield using the photocleavable resin **10**, the Aib synthon **1a**, and the allyl-protected Aib-Pro synthon **2b**. Gly and Leu were introduced with PyBOP as the coupling reagent. Analogously, the syntheses of the hexa- and octapeptides H-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-O^{t}Bu (**21**, A1–A6 of *Trichovirin I 1B*) and H-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu (**22**, A7–A14 of *Trichovirin I 1B*) were carried out. Sequence **21** was chosen to evaluate the introduction of non-aliphatic amino acids, while in the synthesis of **22**, an Aib-Pro unit had to be installed prior to an Aib-residue and another Aib-Pro unit. Both peptides were prepared on the photocleavable resin **10** using the Aib synthon **1a** and the allyl protected Aib-Pro synthon **2b**. All other amino acids were introduced with PyBOP as the coupling reagent. The side chain of Asn was not protected, while the hydroxy group of the serine side chain was masked as benzyl ether, a protecting group which is orthogonal to the ^{t}Bu protecting group. The peptides were obtained in each 34% yield (after prep. HPLC, based on resin loading), which is comparable to that of tetrapeptide **18** and therefore indicating a good linker stability.

Table 1. *Peptides synthesized by means of the 'azirine/oxazolone method' on solid phase.*

Sequence	Description	Yield [%] [a]
H-Ala-Aib-Phe-O^{t}Bu (**15**)	model peptide	33
H-Ala-Aib-Pro-Phe-O^{t}Bu (**18**)	model peptide	35
H-Val-Aib-Gly-Aib-Aib-Pro-Leu-O^{t}Bu (**20**)	A8–A14 of *Trichovirin Ia*	42
H-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-O^{t}Bu (**21**)	A1–A6 of *Trichovirin I 1B*	34
H-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu (**22**)	A7–A14 of *Trichovirin I 1B*	34

[a]) based on resin loading, after prep. HPLC.

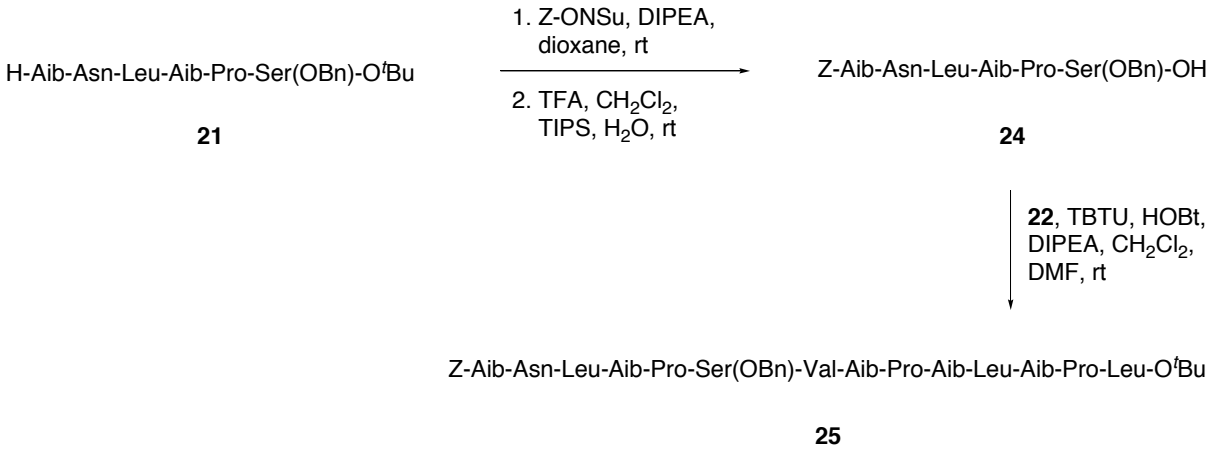
In contrast to the 'PAM/HBr-strategy' (Scheme 1), the use of the photolinker allows the preparation of ^{t}Bu-protected peptides, which, in turn, offers the possibility of segment condensations. For that purpose, the C-terminal ^{t}Bu-protected peptides had to be turned into N-terminal protected peptides. ^{t}Bu-protected peptide **18** was reacted with *N*-(benzyloxycarbonyloxy)succinimid (Z-ONSu), and the corresponding ^{t}Bu protecting group was hydrolyzed to give the N-protected peptide **23** (Scheme 4).



18 **23**

Scheme 4

Analogously, the heptapeptide **21** (A1–A6 of *Trichovirin I 1B*) was turned into the N-protected peptide **24** (Scheme 5). Due to steric hindrance of Aib, the reaction time for the introduction of the Z-protecting group was extended to 8 h. However, the transformation was not complete, and **21** (20%) was partially recovered. The hydrolysis of the corresponding ^{t}Bu ester with TFA was accompanied by partial cleavage of the Aib-Pro amide bond. Most probably, the extent of this side reaction could be reduced by shortening the reaction time (HPLC/MS indicates complete deprotection already after 20 min). Finally, **24** was coupled with **22** (A7–A14 of *Trichovirin I 1B*) and TBTU as the coupling reagent, and protected *Trichovirin I 1B* (Z-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu; **25**) was obtained in 73% yield (Scheme 5).[39]



Scheme 5

3. Conclusions

A method for the direct introduction of Aib-Pro into peptides via 'azirine coupling' on solid phase was developed. The protocol is based on a photocleavable resin (**10**) and the Aib-Pro synthon **2b**. After the coupling of the resin-bound peptide acid with **2b**, the allyl protecting group of the extended peptide was removed by a mild Pd^0-promoted procedure. In contrast to the previously described 'PAM/HBr-strategy', this protocol allows the isolation of peptides with a protected C-terminus. The method found a successful application in the syntheses of different Aib-Pro containing peptaibol segments. A subsequent segment condensation led to the *Trichovirin I 1B* derivative **25**, an oligopeptide containing three Aib-Pro units and two additional Aib residues.

4. Experimental Part

4.1. General

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Aminomethyl polystyrene resin (1% divinylbenzene, 100–200 mesh, loading 1.14 mmol/g) and aminomethyl Tentagel resin (90 μm, loading 0.28 mmol/g) from *Rapp Polymere* (*Rapp Polymere*, Tübingen, Germany). Hydroxymethyl-photolinker AM resin (**10**) (polystyrene, 1% divinylbenzene, 100–200 mesh, loading 0.75 mmol/g) from *Novabiochem* (*Calbiochem-Novabiochem*, Läufelfingen, Switzerland). *N*,2,2-Trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**1a**) was synthesized according to the method of Villalgordo and Heimgartner.[40,41] Methyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2a**), allyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2b**) and phenacyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2c**) were synthesized according to Ref. 9 (**2a**) and Ref. 29 (**2b**, **2c**), respectively. H-Ser(OBn)-O^{t}Bu was prepared by treatment of Fmoc-Ser(OBn)-OH with *tert*-butyl trichloroacetamidate and removal of Fmoc with Et_2NH. The ^{1}H-NMR spectra of H-Ser(OBn)-O^{t}Bu was in accordance with the data given in Ref. 42. Reaction vessels for solid phase synthesis: Single fritted (20 μm) PE reservoir (15 ml) (*Separtis*, Grenzach-Wyhlen, Germany) were wrapped with aluminum foil and used on an *Advanced ChemTech PLS 4 × 6* Shaker (*Advanced ChemTech, Inc.*, Louisville, KY, USA) with a selfmade adapter. The original *Advanced ChemTech* reaction vessels were used for reactions with $COCl_2$. Photolysis was performed in a quartz tube (13.5 cm, 13 mm i.d.). Irradiation with circulary arranged *sterilAir BLB8* lamps (16 × 8 W, λ_{max} = 352 nm) (*sterilAir AG*, Weinfelden, Switzerland). High-

performance liquid chromatography (HPLC): instrument: *Waters 600E* multisolvent delivery system equipped with a *Waters 996 PDA* (*Waters*, Milford, CA, USA); column: *Interchim Uptisphere WOD C18*, 300 Å, 10 μm, 250 × 21.2 mm (*Interchim*, Montluçon, France); eluents: A = H_2O/TFA (0.1%), B = MeCN/TFA (0.1%); flow rate: 10 ml/min; various gradients. Column chromatography (CC): Silica gel *C-560* from *Chemie Uetikon* (*CU Chemie Uetikon GmbH*, Uetikon, Switzerland). IR Spectra: *Perkin-Elmer*, *Spectrum one FT-IR* spectrophotometer (*Perkin-Elmer*, Wellesley, MA, USA), absorptions in cm^{-1}. NMR Spectra: *Bruker AV-600* (*Bruker Biospin*, Karlsruhe, Germany). Chemical shifts in ppm relative to tetramethylsilane as internal standard. 2D-NMR experiments were performed for assignment of the signals. The integer *n* in Xaa^n corresponds to the position of the amino acid within the peptide, but is only given if the amino acid was present more than once in the peptide and if the NMR signal could be assigned unambigously. HPLC/MS: The system consists of a *Rheos 2000* pump, a *Rheos CPS-LC* degasser (*Flux Instruments*, Basel, Switzerland) and a *Thermo Finnigan Surveyor* photo-diode array detector (*Thermo Finnigan*, San Jose, CA, USA). The HPLC-system is equipped with a *HTS PAL* autosampler (*CTC Analytics*, Zwingen, Switzerland) and connected to a *Thermo Finnigan MSQ* linear quadrupole instrument. *Interchim Uptisphere C18-ODB*, 120 Å, 3 μm, 50 × 2.0 mm column; eluents: A = H_2O, B = MeCN, C = HCOOH (1%) in H_2O; flow rate: 0.2 ml/min, gradient (A:B:C): 0.0–15.0 min: 87:3:10–40:50:10. MS: *Bruker ESQUIRE-LC* quadrupole instrument (*Bruker Daltonik GmbH*, Bremen, Germany) or *Finnigan TSQ-700* triple quadrupole instrument (*Finnigan* MAT, San Jose, CA, USA). Direct infusion ESI-MS were performed with a syringe infusion pump at a flow rate of 5 μl/min.

4.2. Abbreviations

Aib: α-aminoisobutyric acid; DIPEA: *N*,*N*-diisopropylethylamine; HOBt: 1-hydroxybenzotriazole; PyBOP: (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TBTU: *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborat; TFA: trifluoroacetic acid; TIPS: triisopropylsilane; Z: benzyloxycarbonyl; Z-ONSu: *N*-[(benzyloxycarbonyl)oxy]succinimid.

4.3. General Procedures 1–7 (*GP 1 – GP 7*)

GP 1: *Attachment of the First Amino Acid*. All manipulations were carried out under N_2. Hydroxymethyl-photolinker AM resin was swollen in THF. After filtration, a solution of $COCl_2$ (**CAUTION**) in toluene (ca. 20%, 10 equiv.) and THF (ca. 0.7 ml/100 mg resin) were added to the resin, which was agitated at rt for 2 h, then washed with THF (2×) and CH_2Cl_2 (2×). In a separate vial, H-Xaa-O^{t}Bu · HCl (4 equiv.) was dissolved in DIPEA (8 equiv.) and CH_2Cl_2 (conc. of H-Xaa-O^{t}Bu · HCl = ca. 0.2M). This mixture was added to the resin and any ammonium salt that occured was removed by filtration. The resin was agitated at rt overnight, then washed with DMF (3×) and CH_2Cl_2 (3×).

GP2: *Removing the ^{t}Bu Protecting Group*. The resin was swollen in CH_2Cl_2. TFA in CH_2Cl_2 (2 ml, 1×15 s, 25%; 2 ml, 1×60 min, 50%) and TIPS (5%, in each case) were added, and the resin was agitated at rt. Then, the resin was washed with CH_2Cl_2 (3×), DMF (2×) and CH_2Cl_2 (3×).

GP3: *Coupling with 2*H*-Azirin-3-amines* **1a**, **2a** *and* **2b**. The corresponding resin was swollen in CH_2Cl_2. A solution of 2*H*-azirin-3-amine (4 equiv.) in CH_2Cl_2 (conc. of 2*H*-azirin-3-amine = ca. 0.2M) was added, the resin was agitated at rt overnight, and then washed with CH_2Cl_2 (3×). Unconsumed 2*H*-azirin-3-amine could easily be recovered.

GP 4: *Hydrolysis of the Terminal Amide*. The resin was swollen in THF. Aq. HCl (ca. 2 ml/100 mg resin, 3M in THF/H_2O, prepared from conc. HCl and THF) was added, and the resin was agitated at rt overnight, then washed with THF (3×), DMF (3×) and CH_2Cl_2 (3×).

GP 5: *Coupling with H-Xaa-O^{t}Bu · HCl*. The resin was swollen in DMF. HOBt (6 equiv.) in DMF, PyBOP (4 equiv.) in DMF, H-Xaa-O^{t}Bu · HCl (4 equiv.) in DMF and DIPEA (12 equiv.) were added (conc. of H-Xaa-O^{t}Bu · HCl = ca. 0.2M), the resin was agitated at rt, and then washed with DMF (3×) and CH_2Cl_2 (3×).

GP 6: *Removal of the Allyl Protecting Group*. The resin was dried i.v. All manipulations were carried out under Ar. The resin was swollen in CH_2Cl_2. A mixture of $Pd(Ph_3P)_4$ (0.3 equiv.) and $PhSiH_3$ (20 equiv.) in CH_2Cl_2 (ca. 2 ml/100 mg resin) was added, the resin was agitated at rt for 1.5 h, and then washed with CH_2Cl_2 (3×), DMF, CH_2Cl_2 (2×), THF (1% H_2O) (2×), THF (1×), MeOH and CH_2Cl_2 (2×).

GP 7: *Cleavage*. The resin (ca. 0.075 mmol) was swollen in a solution of semicarbazide hydrochloride (24 mg) in MeOH/THF (2:1, 6 ml), and the mixture was degassed by bubbling Ar into the mixture. Under vigorous stirring, the resin was irradiated with 16 × 8 W (λ_{max} = 352 nm) for 2 h, then the supernatant solution was removed, and the irradiation process was repeated two times. Afterwards, the resin was washed with MeOH/THF (2:1, 3×). All

solutions were combined, concentrated under reduced pressure, and the crude product was purified by means of HPLC. The purified product was lyophilized.

4.4. Synthesis of Peptides

4.4.1. H-Ala-Aib-Pro-Val-OH (9)

Aminomethyl Tentagel resin (350 mg, 0.098 mmol) was swollen in DMF. 4-(hydroxymethyl)phenylacetic acid (33 mg, 0.199 mmol), DIPEA (103 µl, 0.602 mmol) and PyBOP (104 mg, 0.200 mmol) in DMF (1.5 ml) were added, and the resin was agitated at rt for 30 min (negative Kaiser test), then washed with DMF (3×), CH_2Cl_2 (3×) and THF (3×). The resin was treated as described in *GP 1*, *2*, and *3*. Then, the resin was swollen in a mixture of THF (1 ml) and MeOH (0.7 ml), and LiOH · H_2O (33 mg, 0.787 mmol) in H_2O (0.3 ml) was added. The resin was agitated at rt overnight, washed with THF/MeOH/H_2O (4:3:1, 3×), and DMF (3×). The resin was treated as described in *GP 5* (2 h), then swollen in CH_2Cl_2. HBr in AcOH (33%, 3 ml) and two drops of H_2O were added, and the resin was agitated at rt for 4h. The resin was separated by filtration and washed with AcOH/CH_2Cl_2 (1:1, 3×) and MeCN/CH_2Cl_2 (1:1, 3×). The solvents were evaporated under reduced pressure and the crude product was purified by means of HPLC. The purified product was lyophilized and yielded **9** (10 mg, 21%) as a colorless powder. IR (KBr): 3435s, 3271s, 3068s, 2969s, 2882s, 1674vs, 1630vs, 1548s, 1472m, 1422s, 1369m, 1338w, 1310w, 1269m, 1202vs, 1183vs, 1138vs, 1005w, 979w, 929w, 837w, 800w, 722m. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): ca. 10.0–7.0 (br s, NH_3(Ala)); 8.61 (s, NH(Aib)); 7.73 (d, J = 8.2 Hz, NH(Val)); 4.42–4.41 (m, CH(α)(Pro)); 4.06–4.04 (m, CH(α)(Val)); 3.88–3.87 (m, CH(α)(Ala)); 3.57–3.54, 3.38–3.34 (2m, CH_2(δ)(Pro)); 2.04 (dsept., J = 6.6, 6.6 Hz, CH(β)(Val)); 1.88–1.83, 1.81–1.76 (2m, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.37, 1.34 (2s, 2 Me(Aib)); 1.36 (d, J = 7.6 Hz, Me(Ala)); 0.90, 0.89 (2d, J = 6.8 Hz, 2 Me(Val)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 173.0 (s, CO(Val)); 171.9 (s, CO(Pro)); 170.4 (s, CO(Aib)); 168.4 (s, CO(Ala)); 60.6 (d, CH(α)(Pro)); 57.2 (d, CH(α)(Val)); 56.0 (s, C(α)(Aib)); 47.9 (d, CH(α)(Ala)); 47.6 (t, CH_2(δ)(Pro)); 29.9 (d, CH(β)(Val)); 27.9, 25.2 (2t, CH_2(β)(Pro), CH_2(γ)(Pro)); 24.9, 24.5 (2q, 2 Me(Aib)); 19.1, 18.1 (2q, 2 Me(Val)); 17.3 (q, Me(Ala)). ESI-MS: 371 (100, $[M+H]^+$), 215 (34, [Pro-Val]$^+$).

4.4.2. H-Ala-Aib-Phe-O^{t}Bu (15)

Aminomethyl polystyrene resin (62 mg, 0.071 mmol) was swollen in DMF. 4-[(4-Hydroxymethyl-2-methoxy-5-nitrophenyl)oxy]butanoic acid (40 mg, 0.140 mmol), PyBOP (72 mg, 0.138 mmol) and DIPEA (72 μl, 0.421 mmol) in DMF (1.5 ml) were added, and the resin was agitated at rt for 30 min, then washed with DMF (3×), CH_2Cl_2 (2×) and THF (2×). The resin was treated as described in *GP 1*, *2*, *3*, *4*, *5* (overnight), and *7* to yield **15** (11.4 mg, 33%) as a colorless powder after prep. HPLC purification and lyophilization. IR (KBr): 3426s, 3399s, 3288s, 3065m, 3032m, 2983s, 2939s, 1724s, 1674vs, 1549s, 1516s, 1457m, 1440m, 1393m, 1368s, 1324w, 1259s, 1204vs, 1180vs, 1156vs, 1139vs, 1079w, 1030w, 1015w, 1003w, 948w, 929w, 881w, 839m, 800w, 754w, 739w, 722m, 700m. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): 8.31 (s, NH(Aib)); 8.01 (br s, NH_3(Ala)); 7.67 (d, *J* = 7.8 Hz, NH(Phe)); 7.29–7.19 (m, 5 arom. H); 4.34 (ddd, *J* = 8.2, 7.8, 6.4 Hz, CH(α)(Phe)); 3.84 (q, *J* = 6.9 Hz, CH(α)(Ala)); 3.02 (dd, *J* = 13.8, 6.4 Hz, 1 H of CH_2(Phe)); 2.95 (dd, *J* = 13.8, 8.2 Hz, 1 H of CH_2(Phe)); 1.38, 1.37 (2s, 2 Me(Aib)); 1.33 (s, Me_3C); 1.32 (d, *J* = 7.3 Hz, Me(Ala)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 172.9 (s, CO(Aib)); 170.4 (s, CO(Phe)); 168.8 (s, CO(Ala)); 137.4 (s, arom. C); 129.2, 128.1, 126.4 (3d, 5 arom. CH); 80.7 (s, Me_3*C*); 56.3 (s, C(α)(Aib)); 54.2 (d, CH(α)(Phe)); 48.3 (d, CH(α)(Ala)); 36.8 (t, CH_2(Phe)); 27.5 (q, *Me*$_3$C); 24.7, 24.6 (2q, 2 Me(Aib)); 17.1 (q, Me(Ala)). ESI-MS: 378 (53, [*M*+H]$^+$), 322 (100, [*M*–^{t}Bu]$^+$). HPLC/MS: t_R 10.3 min, *m*/*z* 378 (16, [*M*+H]$^+$), 322 (100, [*M*–^{t}Bu]$^+$), 157 (27, [*M*–(Phe-O^{t}Bu)]$^+$).

4.4.3. H-Ala-Aib-Pro-Phe-O^{t}Bu (18)

Hydroxymethyl-photolinker AM resin (101 mg, 0.075 mmol) was treated as described in *GP 1*, *2*, *3*, *6*, *5* (2 h), and *7* to yield **18** (15.6 mg, 35%) as a colorless powder after prep. HPLC purification and lyophilization. IR (KBr): 3428s, 3293s, 3065s, 3032s, 2983s, 2940s, 1728s, 1674vs, 1548s, 1536s, 1499m, 1472m, 1456m, 1422m, 1415m, 1395m, 1369m, 1258m, 1203vs, 1177vs, 1156vs, 1135vs, 1052w, 1029w, 1004w, 928w, 879w, 836w, 800w, 740w, 721m, 701m. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): 8.65 (s, NH(Aib)); 8.08 (br s, NH_3(Ala)); 7.91 (d, *J* = 7.4 Hz, NH(Phe)); 7.29–7.20 (m, 5 arom. H); 4.33–4.31 (m, CH(α)(Pro)); 4.26 (ddd, *J* = 7.3, 7.3, 7.3 Hz, CH(α)(Phe)); 3.88–3.87 (m, CH(α)(Ala)); 3.52–3.50, 3.38–3.35 (2m, CH_2(δ)(Pro)); 2.97–2.95 (m, CH_2(Phe)); 1.86–1.76, 1.69–1.67 (2m, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.36 (d, *J* = 7.1 Hz, Me(Ala)); 1.35, 1.34 (2s, 2 Me(Aib)); 1.30 (s, Me_3C). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 171.7 (s, CO(Pro)); 170.4 (s, CO(Phe)); 170.3 (s, CO(Aib)); 168.3 (s, CO(Ala)); 137.3 (s, arom. C); 129.2, 128.0, 126.3 (3d, 5 arom.

CH); 80.3 (s, Me_3C); 60.7 (d, CH(α)(Pro)); 55.9 (s, C(α)(Aib)); 54.2 (d, CH(α)(Phe)); 47.8 (d, CH(α)(Ala)); 47.5 (t, CH_2(δ)(Pro)); 36.6 (t, CH_2(Phe)); 27.9 (t, CH_2(β)(Pro)); 27.4 (s, Me_3C); 25.0 (t, CH_2(γ)(Pro)); 24.7, 24.6 (2q, 2 Me(Aib)); 17.1 (q, Me(Ala)). ESI-MS: 497 (10, $[M+Na]^+$), 475 (100, $[M+H]^+$), 419 (17, $[M-{}^t Bu]^+$), 319 (7, $[M-(Ala\text{-}Aib)]^+$). HPLC/MS: t_R 9.9 min, *m/z* 497 (10, $[M+Na]^+$), 475 (30, $[M+H]^+$), 419 (24, $[M-{}^t Bu]^+$), 319 (32, $[M-(Ala\text{-}Aib)]^+$), 263 (100, $[M-(Ala\text{-}Aib)-{}^t Bu]^+$).

4.4.4. H-Val-Aib-Gly-Aib-Aib-Pro-Leu-O^{t}Bu (20)

Hydroxymethyl-photolinker AM resin (100 mg, 0.075 mmol) was treated as described in *GP 1*, *2*, *3*, *4*, *5* (overnight), *2*, *3*, *4*, *3*, *6*, *5* (2 h), and *7* to yield **20** (25.7 mg, 42%) as a colorless powder after prep. HPLC purification and lyophilization. IR (KBr): 3433vs, 3317vs, 3063m, 2978s, 2939s, 2877m, 1725m, 1671vs, 1543vs, 1537vs, 1469m, 1440m, 1416m, 1397m, 1384m, 1368m, 1333w, 1282m, 1248m, 1203vs, 1178vs, 1146s, 1018w, 947w, 878w, 837w, 801w, 722w. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): 8.82 (s, NH(Aib^2)); 8.20 (s, NH(Gly)); 8.08 (br s, NH_3(Val)); 7.81 (d, *J* = 8.1 Hz, NH(Leu)); 7.72 (s, NH(Aib^4)); 7.43 (s, NH(Aib^5)); 4.37–4.35 (m, CH(α)(Pro)); 4.11–4.07 (m, CH(α)(Leu)); 3.61–3.59 (m, CH(α)(Val), 1 H of CH_2(Gly), 1 H of CH_2(δ)(Pro)); 3.55–3.51 (m, 1 H of CH_2(Gly)); 3.43–3.39 (m, 1 H of CH_2(δ)(Pro)); 2.12 (dsept., *J* = 6.7, 6.7 Hz, CH(β)(Val)); 2.02–1.98 (m, 1 H of CH_2(β)(Pro)); 1.74–1.69 (m, 1 H of CH_2(β)(Pro), CH_2(γ)(Pro)); 1.63–1.57 (m, CH(γ)(Leu), 1 H of CH_2(β)(Leu)); 1.51–1.45 (m, 1 H of CH_2(β)(Leu)); 1.403, 1.398 (2s, 2 Me(Aib^2), 2 Me(Aib^4)); 1.38 (s, Me_3C); 1.35, 1.33 (2s, 2 Me(Aib^5)); 0.96, 0.94 (2d, *J* = 7.0 Hz, 2 Me(Val)); 0.89, 0.82 (2d, *J* = 6.3 Hz, 2 Me(Leu)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 174.6 (s, CO(Aib^2)); 174.3 (s, CO(Aib^4)); 171.7 (s, CO(Pro)); 171.1 (s, CO(Leu)); 171.0 (s, CO(Aib^5)); 168.4 (s, CO(Gly)); 168.0 (s, CO(Val)); 79.9 (s, Me_3C); 60.8 (d, CH(α)(Pro)); 57.5 (d, CH(α)(Val)); 56.13 (s, C(α)(Aib^4)); 56.09 (s, C(α)(Aib^2)); 56.0 (s, C(α)(Aib^5)); 50.9 (d, CH(α)(Leu)); 47.2 (t, CH_2(δ)(Pro)); 43.8 (t, CH_2(Gly)); 39.3 (t, CH_2(β)(Leu)); 29.5 (d, CH(β)(Val)); 28.4 (t, CH_2(β)(Pro)); 27.5 (q, Me_3C); 25.8 (q, 1 Me of 2 Me(Aib^4)); 25.6 (q, 1 Me of 2 Me(Aib^2)); 25.2 (q, 1 Me of 2 Me(Aib^5)); 25.0 (t, CH_2(γ)(Pro)); 24.5 (q, 1 Me of 2 Me(Aib^4)); 24.2 (d, CH(γ)(Leu)); 24.0 (q, 1 Me of 2 Me(Aib^5)); 23.7 (q, 1 Me of 2 Me(Aib^2)); 22.7, 21.2 (2q, 2 Me(Leu)); 18.4, 17.5 (2q, 2 Me(Val)). ESI-MS: 718 (17, $[M+Na]^+$), 696 (100, $[M+H]^+$). HPLC/MS: t_R 11.5 min, *m/z* 696 (100, $[M+H]^+$), 640 (27, $[M-{}^t Bu]^+$), 412 (24, $[M-(Pro\text{-}Leu\text{-}O{}^t Bu)]^+$), 339 (16).

4.4.5. H-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-O^{t}Bu (21)

Hydroxymethyl-photolinker AM resin (100 mg, 0.075 mmol) was treated as described in *GP 1*, *2*, *5* (overnight), *2*, *5* (90 min), *2*, *3*, *6*, *5* (90 min), and *7* to yield **21** (21.7 mg, 34%) as a colorless powder after prep. HPLC purification and lyophilization. IR (KBr): 3427s, 3301s, 3066m, 2979m, 2961m, 2938m, 2875m, 1673vs, 1535s, 1470m, 1453m, 1424m, 1412m, 1369m, 1247m, 1203vs, 1182vs, 1138vs, 837w, 800w, 742w, 722m, 700w. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): 8.39–8.37 (m, NH(Asn)); 8.17–8.13 (m, NH(Leu), NH$_3$(Aib^1), NH(Aib^4)); 7.99 (d, *J* = 8.0 Hz, NH(Ser)); 7.42–7.41 (m, 1 H of CONH$_2$(Asn)); 7.36–7.32, 7.29–7.27 (2m, 5 arom. H); 6.98 (s, 1 H of CONH$_2$(Asn)); 4.70 (ddd, *J* = 7.5, 7.5, 7.5 Hz, CH(α)(Asn)); 4.49, 4.53 (AB, *J* = 12.1 Hz, OC*H*$_2$Ph(Ser)); 4.42–4.40 (m, CH(α)(Pro)); 4.36–4.33 (m, CH(α)(Ser)); 4.29–4.25 (m, CH(α)(Leu)); 3.72 (dd, *J* = 9.7, 5.8 Hz, 1 H of CH$_2$(β)(Ser)); 3.65 (dd, *J* = 9.7, 4.4 Hz, 1 H of CH$_2$(β)(Ser)); 3.46–3.44, 3.42–3.38 (2m, CH$_2$(δ)(Pro)); 2.64–2.60, 2.43–2.39 (2m, CH$_2$(β)(Asn)); 1.90–1.87, 1.77–1.72 (2m, CH$_2$(β)(Pro), CH$_2$(γ)(Pro)); 1.60–1.55 (m, CH(γ)(Leu)); 1.49–1.43 (m, CH$_2$(β)(Leu)); 1.45, 1.43 (2s, 2 Me(Aib^1)); 1.37 (s, Me$_3$C); 1.34, 1.30 (2s, 2 Me(Aib^4)); 0.86, 0.80 (2d, *J* = 6.6 Hz, 2 Me(Leu)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 171.9 (s, CO(Pro)); 171.2 (s, CO(Leu), CONH$_2$(Asn)); 171.1 (s, CO(Aib^1)); 170.8 (s, CO(Aib^4)); 170.3 (s, CO(Asn)); 169.0 (s, CO(Ser)); 137.9 (s, arom. C); 128.1, 127.5, 127.4 (3d, 5 arom. CH); 80.5 (s, Me$_3$*C*); 72.1 (t, O*C*H$_2$Ph(Ser)); 69.3 (t, CH$_2$(β)(Ser)); 60.5 (d, CH(α)(Pro)); 56.2 (s, C(α)(Aib^1)); 55.6 (s, C(α)(Aib^4)); 52.8 (d, CH(α)(Ser)); 50.8 (d, CH(α)(Leu)); 49.8 (d, CH(α)(Asn)); 47.3 (t, CH$_2$(δ)(Pro)); 40.3 (t, CH$_2$(β)(Leu)); 36.8 (t, CH$_2$(β)(Asn)); 28.0 (t, CH$_2$(β)(Pro)); 27.5 (q, *Me*$_3$C); 25.0 (t, CH$_2$(γ)(Pro)); 24.9, 24.7 (2q, 2 Me(Aib^4)); 24.1 (d, CH(γ)(Leu)); 23.3, 23.1 (2q, 2 Me(Aib^1)); 22.9, 21.3 (2q, 2 Me(Leu)). ESI-MS: 768 (100, [*M*+Na]$^+$), 746 (48, [*M*+H]$^+$), 712 (28, [*M*–^{t}Bu+Na]$^+$), 622 (17). HPLC/MS: t_R 12.4 min, *m*/*z* 746 (100, [*M*+H]$^+$), 398 (34, [*M*–(Pro-Ser(OBn)-^{t}Bu)]$^+$).

4.4.6. H-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu (22)

Hydroxymethyl-photolinker AM resin (100 mg, 0.075 mmol) was treated as described in *GP 1*, *2*, *3*, *6*, *3*, *4*, *5* (overnight), *2*, *3*, *6*, *5* (2 h), and *7* to yield **22** (24.8 mg, 34%) as a colorless powder after prep. HPLC purification and lyophilization. IR (KBr): 3440vs, 3330sh, 3058m, 2961s, 2874m, 1724sh, 1653vs, 1627vs, 1536vs, 1471s, 1451m, 1440m, 1416s, 1385m, 1368m, 1344w, 1249m, 1241m, 1203vs, 1178vs, 1146s, 839w, 833w, 800w, 722w. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): 9.00 (s, NH(Aib^2)); 8.13 (br s, NH$_3$(Val)); 7.77 (d, *J* = 7.8 Hz, NH(Leu^8)); 7.72 (s, NH(Aib^4)); 7.69 (s, NH(Aib^6)); 7.38 (d, *J* = 8.5 Hz, NH(Leu^5));

4.37–4.35 (m, CH(α)(Pro^7)); 4.17–4.12 (m, CH(α)(Leu^5), CH(α)(Pro^3)); 4.04–4.00 (m, CH(α)(Leu^8)); 3.76–3.73 (m, CH(α)(Val)); 3.60–3.55 (m, 3 H of 2 CH_2(δ)(Pro)); 3.40–3.36 (m, 1 H of 2 CH_2(δ)(Pro)); 2.21–2.18 (m, CH(β)(Val)); 2.12–2.08 (m, 1 H of 2 CH_2(β)(Pro)); 2.00–1.95 (m, 1 H of 2 CH_2(β)(Pro), 1 H of 2 CH_2(γ)(Pro)); 1.89–1.85 (m, 1 H of 2 CH_2(γ)(Pro)); 1.74–1.68 (m, 2 H of 2 CH_2(β)(Pro), 2 H of 2 CH_2(γ)(Pro)); 1.66–1.57 (m, 3 H of 2 CH_2(β)(Leu), 2 CH(γ)(Leu)); 1.48–1.44 (m, 1 H of 2 CH_2(β)(Leu)); 1.42, 1.40 (2s, 2 Me of 6 Me(Aib)); 1.38 (s, Me_3C, 1 Me of 6 Me(Aib)); 1.36, 1.35 (2s, 3 Me of 6 Me(Aib)); 1.00, 0.94 (2d, *J* = 7.0 Hz, 2 Me(Val)); 0.91, 0.87, 0.82, 0.78 (4d, *J* = 6.2 Hz, 4 Me(Leu)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 173.9 (s, CO(Aib^4)); 172.6 (s, CO(Pro^3)); 172.2 (s, CO(Aib^2)); 171.7 (s, CO(Leu^5), CO(Pro^7)); 171.3 (s, CO(Leu^8)); 170.8 (s, CO(Aib^6)); 167.5 (s, CO(Val)); 79.9 (s, Me_3C); 63.2 (d, CH(α)(Pro^3)); 60.8 (d, CH(α)(Pro^7)); 56.9 (d, CH(α)(Val)); 56.2 (s, C(α)(Aib^4)); 56.1 (s, C(α)(Aib^2)); 55.6 (s, C(α)(Aib^6)); 51.1 (s, 2 CH(α)(Leu)); 48.0, 47.5 (2t, 2 CH_2(δ)(Pro)); 39.6, 39.2 (2t, 2 CH_2(β)(Leu)); 29.6 (d, CH(β)(Val)); 28.3, 27.6 (2t, 2 CH_2(β)Pro)); 27.5 (q, Me_3C); 26.4 (q, 1 Me of 6 Me(Aib)); 25.5 (t, 1 CH_2 of 2 CH_2(γ)(Pro)); 25.4 (q, 1 Me of 6 Me(Aib)); 25.1 (t, 1 CH_2 of 2 CH_2(γ)(Pro)); 24.6, 24.32, 24.29 (3q, 3 Me of 6 Me(Aib)); 24.27, 24.2 (2d, 2 CH(γ)(Leu)); 23.9 (q, 1 Me of 6 Me(Aib)); 23.0, 22.9, 21.2, 20.6 (4q, 4 Me(Leu)); 18.6, 16.7 (2q, 2 Me(Val)). ESI-MS: 871 (19, [*M*+Na]$^+$), 849 (100, [*M*+H]$^+$). HPLC/MS: t_R 13.9 min, *m*/*z* 849 (100, [M+H]$^+$), 565 (93, [*M*–(Pro-Leu-O^{t}Bu)]$^+$).

4.4.7. Z-Ala-Aib-Pro-Phe-OH (23)

Z-ONSu (4.3 mg, 17.3 μmol) and DIPEA (8.1 μl, 47.3 μmol) were added to a solution of **18** (9.3 mg, 15.8 μmol) in dioxane (2.5 ml) at rt, and the solution was stirred at rt for 3 h. The mixture was concentrated, and the crude product was purified by prep. HPLC. After lyophilization, Z-Ala-Aib-Pro-Phe-O^{t}Bu (HPLC/MS: t_R 16.5 min, *m*/*z* 631 (100, [*M*+Na]$^+$)) (8.5 mg, 89%) was obtained as a colorless powder, which was dissolved in CH_2Cl_2/TFA (1:1, 2 ml) and TIPS (100 μl). The solution was stirred at rt for 1 h, then it was concentrated under reduced pressure, and the crude product was purified by prep. HPLC. The purified product was lyophilized and yielded **23** (7.5 mg, 97%) as a colorless powder. IR (KBr): 3412vs, 3292vs, 3063m, 3032m, 2984s, 2940m, 2876m, 1722vs, 1650vs, 1535vs, 1499s, 1469m, 1454s, 1411s, 1380m, 1366m, 1340m, 1328m, 1317m, 1282m, 1243vs, 1215s, 1203s, 1177s, 1116m, 1072m, 1040m, 1028m, 1002w, 977w, 911w, 824w, 743m, 700s. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): ca. 13.2–11.2 (br s, COOH); 8.20 (s, NH(Aib)); 7.77 (d, *J* = 8.0 Hz, NH(Phe)); 7.44 (d, *J* = 7.6 Hz, NH(Ala)); 7.38–7.18 (m, 10 arom. H); 5.01, 5.06 (AB, *J* = 12.6 Hz, CH_2(carbamate)); 4.37 (ddd, *J* = 8.4, 8.4, 5.3 Hz, CH(α)(Phe)); 4.29–4.27 (m,

CH(α)(Pro)); 4.10 (dq, *J* = 7.2, 7.2 Hz, CH(α)(Ala)); 3.54–3.50, 3.35–3.31 (2m, CH_2(δ)(Pro)); 3.05 (dd, *J* = 13.9, 5.1 Hz, 1 H of CH_2(Phe)); 2.93 (dd, *J* = 13.9, 9.2 Hz, 1 H of CH_2(Phe)); 1.79–1.73 (m, 1 H of CH_2(β)(Pro)); 1.61–1.58 (m, CH_2(γ)(Pro)); 1.51–1.48 (m, 1 H of CH_2(β)(Pro)); 1.30, 1.29 (2s, 2 Me(Aib)); 1.21 (d, *J* = 7.1 Hz, Me(Ala)). ^{13}C-NMR ((D_6)DMSO, 150 MHz): 172.6 (s, CO(Phe)); 171.9 (s, CO(Ala)); 171.7 (s, CO(Pro)); 170.9 (s, CO(Aib)); 155.6 (s, CO(carbamate)); 137.6, 137.0 (2s, 2 arom. C); 129.1, 128.2, 128.0, 127.7, 127.6, 126.2 (6d, 10 arom. CH); 65.2 (t, CH_2(carbamate)); 60.9 (d, CH(α)(Pro)); 55.5 (s, C(α)(Aib)); 53.2 (d, CH(α)(Phe)); 49.9 (d, CH(α)(Ala)); 47.2 (t, CH_2(δ)(Pro)); 36.6 (t, CH_2(Phe)); 27.8 (t, CH_2(β)(Pro)); 25.0 (q, 1 Me of 2 Me(Aib)); 24.9 (t, CH_2(γ)(Pro)); 24.6 (q, 1 Me of 2 Me(Aib)); 17.9 (q, Me(Ala)). ESI-MS: 591 (67, $[M+K]^+$), 575 (100, $[M+Na]^+$), 553 (44, $[M+H]^+$), 263 (96, $[M–(Pro\text{-}Phe)–CO]^+$, $[Pro\text{-}Phe]^+$). HPLC/MS: t_R 14.0 min, *m*/*z* 591 (51, $[M+K]^+$), 263 (100, $[M–(Pro\text{-}Phe)–CO]^+$, $[Pro\text{-}Phe]^+$).

4.4.8. Z-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-OH (24)

Z-ONSu (6.0 mg, 24.1 μmol) and DIPEA (11.6 μl, 67.8 μmol) were added to a solution of **21** (19.4 mg, 22.6 μmol) in MeCN (5 ml) at rt, and the solution was stirred at rt for 6 h. Additional Z-ONSu (0.6 mg, 2.3 μmol) and DIPEA (3.9 μl, 22.6 μmol) were added, and the solution was stirred at rt for further 2 h. Then, the mixture was concentrated, and the crude product was purified by prep. HPLC. After lyophilization, Z-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-O^{t}Bu (HPLC/MS: t_R 16.6 min, *m*/*z* 880 (62, $[M+H]^+$)) (13.8 mg, 70%; additionally, **21** (3.8 mg, 20%) was isolated) was obtained as a colorless powder, which was dissolved in CH_2Cl_2/TFA (1:1, 2 ml), H_2O (1 drop) and TIPS (100 μl). The solution was stirred at rt for 45 min, then it was concentrated under reduced pressure, and the crude product was purified by prep. HPLC. The purified product was lyophilized and yielded **24** (8.9 mg, 67%) as a colorless powder. IR (KBr): 3418sh, 3308vs, 3063m, 3033m, 2957m, 2872m, 1660vs, 1532vs, 1469s, 1454s, 1412s, 1387m, 1366m, 1267s, 1203s, 1176s, 1094s, 1078m, 1028w, 740m, 698m. ^{1}H-NMR ((D_6)DMSO, 600 MHz): ca. 13.2–11.3 (br s, COOH); 8.49 (d, *J* = 6.0 Hz, NH(Asn)); 8.06 (s, NH(Aib^1)); 7.96 (d, *J* = 7.9 Hz, NH(Ser)); 7.74 (d, *J* = 8.5 Hz, NH(Leu)); 7.49 (s, 1 H of $CONH_2$(Asn)); 7.48 (s, NH(Aib^4)); 7.40–7.26 (m, 10 arom. H); 7.04 (s, 1 H of $CONH_2$(Asn)); 5.08, 5.00 (AB, *J* = 12.4 Hz, CH_2(carbamate)); 4.51 (s, OCH_2Ph(Ser)); 4.43–4.38 (m, CH(α)(Ser), CH(α)(Pro)); 4.32–4.29 (m, CH(α)(Asn)); 4.25–4.21 (m, CH(α)(Leu)); 3.75 (dd, *J* = 9.8, 5.9 Hz, 1 H of CH_2(β)(Ser)); 3.68 (dd, *J* = 9.8, 4.2 Hz, 1 H of CH_2(β)(Ser)); 3.45–3.42 (m, CH_2(δ)(Pro)); 2.67–2.63, 2.57–2.54 (2m, CH_2(β)(Asn)); 1.90–1.86, 1.75–1.56 (2m, CH_2(β)(Pro), CH_2(γ)(Pro), CH_2(β)(Leu),

CH(γ)(Leu)); 1.36 (s, 1 Me of 2 Me(Aib^1)); 1.35, 1.332 (2s, 2 Me(Aib^4)); 1.325 (s, 1 Me of 2 Me(Aib^1)); 0.81, 0.74 (2d, *J* = 6.3 Hz, 2 Me(Leu)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 175.3 (s, CO(Aib^1)); 172.1 (s, $CONH_2$(Asn)); 172.0 (s, CO(Pro)); 171.4 (s, CO(Ser)); 171.2 (s, CO(Leu)); 170.9 (s, CO(Asn)); 170.8 (s, CO(Aib^4)); 156.2 (s, CO(carbamate)); 138.1 (s, arom. C(Bn)); 136.4 (s, arom. C(Z)); 128.4, 128.2, 128.0, 127.7, 127.5, 127.4 (6d, 10 arom. CH); 72.2 (t, O*C*H_2Ph(Ser)); 69.4 (t, CH_2(β)(Ser)); 65.7 (t, CH_2(carbamate)); 60.7 (d, CH(α)(Pro)); 56.1 (s, C(α)(Aib^1)); 55.8 (s, C(α)(Aib^4)); 52.4 (d, CH(α)(Ser)); 51.6 (d, CH(α)(Asn)); 50.9 (d, CH(α)(Leu)); 47.5 (t, CH_2(δ)(Pro)); 39.4 (t, CH_2(β)(Leu)); 35.1 (t, CH_2(β)(Asn)); 27.9 (t, CH_2(β)(Pro)); 26.1 (q, 1 Me of 2 Me(Aib^1)); 25.1 (q, 1 Me of 2 Me(Aib^4)); 25.0 (t, CH_2(γ)(Pro)); 24.8 (q, 1 Me of 2 Me(Aib^4)); 24.0 (d, CH(γ)(Leu)); 23.9 (q, 1 Me of 2 Me(Aib^1)); 23.1, 20.7 (2q, 2 Me(Leu)). ESI-MS: 868 (45, [*M*–H+2Na]$^+$), 846 (100, [*M*+Na]$^+$). HPLC/MS: t_R 15.6 min, *m*/*z* 824 (11, [*M*+H]$^+$), 532 (100, [*M*–(Pro-Ser(OBn)-O^{t}Bu)]$^+$), 447 (40, [*M*–(Aib-Pro-Ser(OBn)-O^{t}Bu)]$^+$).

4.4.9. Z-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu (25).

HOBt, TBTU, and DIPEA were taken from stock-solutions. HOBt (1.7 mg, 11.1 μmol), TBTU (3.5 mg, 11.0 μmol), DIPEA (5.4 μl, 31.3 μmol) and **22** (10.6 mg, 11.0 μmol) were added to a solution of **24** (8.6 mg, 10.4 μmol) in CH_2Cl_2/DMF (1:1, 3 ml). The mixture was stirred at rt for 4 h, the solvent was evaporated under reduced pressure, and the crude product was purified by prep. HPLC. After lyophilization, **25** (12.5 mg, 73%) was obtained as a colorless powder. IR (KBr): 3435sh, 3306s, 2958m, 2872w, 1648vs, 1623vs, 1536vs, 1470s, 1454m, 1412s, 1385m, 1366m, 1269m, 1203m, 1172s, 1152m, 1094w, 1028w, 740w, 698w. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): 8.57 (d, *J* = 5.5 Hz, NH(Asn)); 8.11 (s, 1 NH of 5 NH(Aib)); 7.90 (d, *J* = 6.7 Hz, NH(Ser)); 7.82 (d, *J* = 7.7 Hz, 1 NH of 3 NH(Leu)); 7.82, 7.79 (2s, 2 NH of 5 NH(Aib)); 7.74 (d, *J* = 7.7 Hz, 1 NH of 3 NH(Leu)); 7.55, 7.54 (2s, 2 NH of 5 NH(Aib)); 7.53 (s, 1 H of $CONH_2$(Asn)); 7.39–7.30, 7.27–7.24 (2m, 10 arom. H, 1 NH of 3 NH(Leu), NH(Val)); 7.07 (s, 1 H of $CONH_2$(Asn)); 5.07, 5.00 (AB, *J* = 12.3 Hz, CH_2(carbamate)); 4.55, 4.51 (AB, *J* = 11.9 Hz, OC*H*$_2$Ph(Ser)); 4.38–4.36 (m, 1 H of 3 CH(α)(Pro)); 4.33–4.26 (m, CH(α)(Ser), CH(α)(Asn), 1 H of 3 CH(α)(Leu), 1 H of 3 CH(α)(Pro)); 4.19–4.14 (m, 1 H of 3 CH(α)(Leu), CH(α)(Val)); 4.08 (dd, J = 8.4, 8.4 Hz, 1 H of 3 CH(α)(Pro)); 4.04–4.00 (m, 1 H of 3 CH(α)(Leu)); 3.86–3.83 (m, 1 H of CH_2(β)(Ser)); 3.77–3.68 (m, 1 H of CH_2(β)(Ser), 2 H of 3 CH_2(δ)(Pro)); 3.65–3.61, 3.52–3.48, 3.38–3.34, 3.30–3.25 (4m, 4 H of 3 CH_2(δ)(Pro)); 2.68–2.64, 2.60–2.56 (2m, CH_2(β)(Asn)); 2.24–2.19 (m, 2 H of 3 CH_2(β)(Pro)); 2.09 (dsept., *J* = 6.8, 6.8 Hz, CH(β)(Val)); 2.01–1.98 (m, 1 H of 3

CH_2(β)(Pro)); 1.93–1.83 (m, 4 H of 3 CH_2(γ)(Pro)); 1.74–1.57 (m, 3 CH(γ)(Leu), 4 H of 3 CH_2(β)(Leu), 3 H of 3 CH_2(β)(Pro), 2 H of 3 CH_2(γ)(Pro)); 1.54–1.51 (m, 1 H of 3 CH_2(β)(Leu)); 1.48 (s, 1 Me of 10 Me(Aib)); ca. 1.44 (m, 1 H of 3 CH_2(β)(Leu)); 1.43, 1.42, 1.38, 1.37, 1.364, 1.358, 1.34 (7s, 9 Me of 10 Me(Aib), Me_3C); 0.91 (d, *J* = 6.4 Hz, 1 Me of 6 Me(Leu)); 0.87, 0.83 (2d, *J* = 6.8 Hz, 2 Me(Val)); 0.82 (d, *J* = 6.4 Hz, 2 Me of 6 Me(Leu)); 0.749, 0.745 (2d, *J* = 6.0 Hz, 2 Me of 6 Me(Leu)); 0.63 (d, *J* = 6.2 Hz, 1 Me of 6 Me(Leu)). ^{13}C-NMR ((D_6)DMSO, 150 MHz): 175.7, 174.2, 173.0. 172.8, 172.74, 172.70, 172.6, 171.90, 171.86, 171.6, 171.3, 171.2, 171.1, 170.9, 169.6 (15s, 15 CO); 156.2 (s, CO(carbamate)); 137.9 (s, arom. C of CH_2Ph(Ser)); 136.2 (s, arom. C(Z)); 128.3, 128.1, 127.9, 127.6, 127.4, 127.2 (6d, 10 arom. CH); 79.8 (s, Me_3C); 72.0 (t, OCH_2Ph(Ser)); 68.6 (t, CH_2(β)(Ser)); 65.8 (t, CH_2(carbamate)); 63.9, 62.9, 60.8 (3d, 3 CH(α)(Pro)); 58.3 (d, CH(α)(Val)); 56.1, 56.0, 55.9, 55.8, 55.6 (5s, 5 C(α)(Aib)); 55.4, 51.9 (2d, CH(α)(Asn), CH(α)(Ser)); 51.1, 51.0 (2d, 3 CH(α)(Leu)); 48.3, 48.2, 47.6 (3t, 3 CH_2(δ)(Pro)); 39.5, 39.1, 39.1 (3t, 3 CH_2(β)(Leu)); 34.9 (t, CH_2(β)(Asn)); 29.6 (d, CH(β)(Val)); 28.4, 28.3, 28.1 (3t, 3 CH_2(β)(Pro)); 27.5 (q, Me_3C); 26.5, 26.0, 25.7 (3q, 3 Me of 10 Me(Aib)); 25.6 (t, 1 CH_2 of 3 CH_2(γ)(Pro)); 25.5 (q, 1 Me of 10 Me(Aib)); 25.4 (t, 1 CH_2 of 3 CH_2(γ)(Pro)); 25.3 (q, 1 Me of 10 Me(Aib)); 25.1 (t, 1 CH_2 of 3 CH_2(γ)(Pro)); 24.2 (q,d, 1 Me of 10 Me(Aib), 2 CH of 3 CH(γ)(Leu)); 23.81 (q, 1 Me of 10 Me(Aib)); 23.76 (d, 1 CH of 3 CH(γ)(Leu)); 23.3 (q, 2 Me of 10 Me(Aib)); 23.1 (q, 1 Me of 10 Me(Aib)); 23.0, 22.93, 22.89, 21.1, 20.3, 20.2 (6q, 6 Me(Leu)); 18.8, 17.9 (2q, 2 Me(Val)). ESI-MS: 1693 (17, $[M+K]^+$), 1677 (85, $[M+Na]^+$). HPLC/MS: t_R 18.1 min, *m*/*z* 1676 (2, $[M+H]^+$), 846 (100), 819 (35).

References

1. H. Heimgartner, *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 238.
2. P. Wipf, Ph.D. thesis, Universität Zürich, 1987.
3. P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1988**, *71*, 140.
4. P. Wipf, H. Heimgartner, *Helv. Chim. Acta* **1990**, *73*, 13.
5. C. Strässler, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **1997**, *80*, 1528.
6. G. Suter, S. A. Stoykova, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2000**, *83*, 2961.
7. S. Stamm, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2003**, *86*, 1371.
8. W. Altherr, H. Heimgartner, in 'Peptides 1990', Eds. E. Giralt, D. Andreu, ESCOM, Leiden, 1991, p. 107.
9. R. Luykx, C. B. Bucher, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **1996**, *79*, 527.
10. C. B. Bucher, H. Heimgartner, *Helv. Chim. Acta* **1996**, *79*, 1903.
11. N. Pradeille, H. Heimgartner, *J. Pept. Sci.* **2003**, *9*, 827.
12. R. T. N. Luykx, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2003**, *86*, 4093.
13. N. Pradeille, O. Zerbe, K. Möhle, A. Linden, H. Heimgartner, *Chem. Biodiv.* **2005**, *2*, 1127.
14. J. Lehmann, A. Linden, H. Heimgartner, *Tetrahedron* **1998**, *54*, 8721.
15. J. Lehmann, H. Heimgartner, *Helv. Chim. Acta* **1999**, *82*, 1899.
16. R. Breitenmoser, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2002**, *85*, 990.
17. T. Jeremic, A. Linden, H. Heimgartner, *Chem. Biodiv.* **2004**, *1*, 1730.
18. T. Jeremic, A. Linden, K. Möhle, H. Heimgartner, *Tetrahedron* **2005**, *61*, 1871.
19. J. M. Villalgordo, H. Heimgartner, *Helv. Chim. Acta* **1997**, *80*, 748.
20. K. N. Koch, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2000**, *83*, 233.
21. K. N. Koch, H. Heimgartner, *Helv. Chim. Acta* **2000**, *83*, 1881.
22. B. Iliev, A. Linden, R. Kunz, H. Heimgartner, *Tetrahedron* **2006**, *62*, 1079.
23. P. Köttgen, A. Linden, H. Heimgartner, *Helv. Chim. Acta*, in press.
24. S. Stamm, H. Heimgartner, *Eur. J. Org. Chem.* **2004**, 3820.
25. S. Stamm, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2006**, *89*, 1.
26. H. Brückner, T. Kripp, M. Kiess, in 'Peptides 1990', Eds. E. Giralt, D. Andreu, ESCOM, Leiden, 1991, p. 347.
27. C. Auvin-Guette, S. Rebuffat, Y. Prigent, B. Bodo, *J. Am. Chem. Soc.* **1992**, *114*, 2170.
28. M. K. Das, S. Raghothamma, P. Balaram, *Biochemistry* **1986**, *25*, 7110.
29. S. Stamm, H. Heimgartner, *Helv. Chim. Acta*, submitted.
30. H. Kunz, B. Dombo, *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 711.

31. M. Hiersemann, *Tetrahedron* **1999**, *55*, 2625.
32. K. Kaljuste, A. Undén, *Tetrahedron Lett.* **1996**, *37*, 3031.
33. K. C. Nicolaou, N. Petasis, S. Seitz, *J. Chem. Soc., Chem. Comm.* **1981**, 1195.
34. C. P. Holmes, D. G. Jones, *J. Org. Chem.* **1995**, *60*, 2318.
35. C. P. Holmes, *J. Org. Chem.* **1997**, *62*, 2370.
36. For a review about photolabile protecting groups and linkers, see: C. G. Bochet, *J. Chem. Soc., Perkin Trans. 1*, **2002**, 125.
37. A. Patchornik, B. Amit, R. B. Woodward, *J. Am. Chem. Soc.* **1970**, *92*, 6333.
38. M. Dessolin, M.-G. Guillerez, N. Thieriet, F. Guibé, A. Loffet, *Tetrahedron Lett.* **1995**, *36*, 5741.
39. For solution phase syntheses of *Trichovirin I*, see: H. Brückner, A. Koza, *Amino Acids* **2003**, *24*, 311 and ref. 12.
40. J. M. Villalgordo, H. Heimgartner, *Tetrahedron* **1993**, *49*, 7215.
41. J. M. Villalgordo, H. Heimgartner, *Helv. Chim. Acta* **1993**, *76*, 2830.
42. P. Strazzolini, T. Melloni, A. G. Giumanini, *Tetrahedron* **2001**, *57*, 9033.

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